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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 31/59

A1

(11) International Publication Number: **WO 99/49870**

(43) International Publication Date: 7 October 1999 (07.10.99)

(21) International Application Number: PCT/US99/06442

(22) International Filing Date: 25 March 1999 (25.03.99)

(30) Priority Data:
60/079,696 27 March 1998 (27.03.98) US

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(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
With amended claims and statement.

(54) Title: VITAMIN D AND ITS ANALOGS IN THE TREATMENT OF TUMORS AND OTHER HYPERPROLIFERATIVE DISORDERS

(57) Abstract

Treatment of hyperproliferative disorders (including tumors and psoriasis) by pulse administration of a drug (such as Vitamin D or an analog) that increases blood or tissue levels of Vitamin D. The drug is administered at a sufficient dose to have an anti-proliferative effect, but the pulsed administration of the drug avoids the development of severe symptomatic or life-threatening hypercalcemia. In particular embodiments, avoidance of hypercalcemia (as measured by serum levels of calcium above normal range) is avoided altogether. In a particular example, the drug is calcitriol administered at an oral dose of about 0.5 mcg/kg once a week.

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VITAMIN D AND ITS ANALOGS IN THE TREATMENT OF TUMORS AND OTHER HYPERPROLIFERATIVE DISORDERS

FIELD OF THE INVENTION

This invention concerns the use of Vitamin D and its analogs in the treatment of tumors and hyperproliferative disorders.

BACKGROUND OF THE INVENTION

Vitamin D is a generic term for a family of secosteroids that have affinity for the Vitamin D receptor, and are involved in the physiologic regulation of calcium and phosphate metabolism. Exposure to the sun and dietary intake are common sources of Vitamin D, but deficiencies of this vitamin can cause rickets and osteomalacia. Supplementation of dairy and other food products has reduced the incidence of Vitamin D deficiency conditions in modern society, and medical research concerning this vitamin has turned to its therapeutic effects in a variety of pathological conditions.

Vitamin D_3 is synthesized in human skin from 7-dehydrocholesterol and ultraviolet light. Vitamin D_3, or its analog Vitamin D_2, can be ingested from the diet, for example in fortified milk products. Vitamin D_2 and D_3 undergo hydroxylation first in the liver to 25-hydroxyvitamin D, then in the kidney to 1α,25-dihydroxycholecalciferol (also known as 1,25-dihydroxyvitamin D or calcitriol), which is the principal biologically active form of Vitamin D. The biological production of this active form of the vitamin is tightly physiologically regulated.

Vitamin D exerts its calcium regulating activity through both genomic and nongenomic pathways. Although the nongenomic pathways remain poorly characterized, the genomic responses are mediated through binding to the nuclear Vitamin D receptor (VDR). The VDR is a ligand-activated transcription factor, which binds the Vitamin D_3 response element contained within the promoter/enhancer region of target genes. Vitamin D maintains calcium levels in the normal range by stimulating intestinal calcium absorption. When intestinal absorption is unable to maintain calcium homeostasis, Vitamin D stimulates monocytic cells to become mature osteoclasts, which in turn mobilize calcium from bones.

Appreciation for Vitamin D's non calcium-related biological activities began in 1979 with Stumpf's discovery that radioactive Vitamin D localizes to many tissues not associated with calcium metabolism (*Science* 206:1188-1190, 1979). In 1981, Abe et al. reported that mouse myeloid leukemia cells possessed VDR, and that their exposure to Vitamin D led to terminal differentiation (*PNAS USA* 78:4990-4994, 1981). Since then VDR has been described in carcinomas of the prostate, breast, colon, lung, pancreas, endometrium, bladder, cervix, ovaries, squamous cell carcinoma, renal cell carcinoma, myeloid and lymphocytic leukemia, medullary thyroid carcinoma, melanoma, multiple myeloma, retinoblastoma, and sarcomas of the soft tissues and bone.

In vitro assays using 1,25 dihydroxyvitamin D or its analogues demonstrated antiproliferative effects in cell lines derived from many malignancies including adenocarcinomas of the prostate (*Molec. and Cell. Endocrinology* 126:83-90, 1997; *Proc. Amer. Assoc. Cancer Res.*

38:456, 1997; *J. Ster. Biochem. and Molec. Biol.* 58:277-288, 1996; *Endocrinology* 137:1554-1561, 1996; *Endocrinology* 136:20-26, 1995; *Cancer Research* 54:805-810, 1994; *Endocrinology* 132:1952-1960, 1993; and *Anticancer Research* 14:1077-1081, 1994), breast (*Proc. Amer. Assoc. Cancer Res.*38:456, 1997; *Biochemical Pharmacology* 44:693-702, 1992); colon (*Biochemical and Biophysical Research Communications* 179:57-62, 1991; *Archives of Pharmacology* 347:105-110, 1993); pancreas (*British Journal of Cancer* 73:1341-1346, 1996); and endometrium (*Journal of Obstetrics and Gynaecology Research* 22:529-539, 1996); lung (*Anticancer Research* 16:2953-2659, 1996); myeloid leukemia (*PNAS USA* 78:4990-4994, 1981); melanoma (*Endocrinology* 108:1083-1086, 1981); and sarcomas of the soft tissues (*Annals of Surgical Oncology* 3:144-149, 1996) and bone (*Journal of the Japanese Orthopaedic Association* 69:181-190, 1995).

Studies in animals have shown antiproliferative activity of Vitamin D or its analogues in prostate cancer (*Urology* 46:365-369, 1994); breast cancer (*J. NCI* 89:212-218, 1997; *Lancet* 1:188-191, 1989); squamous cell carcinoma (*Molecular and Cellular Differentiation* 3:31-50, 1995); myeloid leukemia (Blood 74:82-93, 1989 and *PNAS USA* 80:201-204, 1983) and retinoblastoma (*Archives of Opthalmology* 106:541-543, 1988; *Archives of Opthalmology* 106:536-540, 1988). The mechanism of Vitamin D's antiproliferative effects remains unknown, although it has been proposed that Vitamin D increases synthesis of TGF-β1 and TGF-β2, decreases the expression of epidermal growth factor receptors, leads to dephosphorylation of the retinoblastoma protein, induces cell cycle arrest in G1, perhaps by induction of the cyclin dependent kinase inhibitors p21(waf1) and p27(kip1), and induces the production of insulin-like growth factor binding protein.

The patent literature is replete with attempts to treat tumors with Vitamin D compounds. U.S. Patent No. 4,391,802 disclosed treating leukemioid diseases with 1α-hydroxy Vitamin D derivatives. The use of 1α-hydroxy derivatives with a 17 side chain greater in length than the cholesterol or ergosterol side chains was disclosed in U.S. Patent No. 4,717,721. Additional Vitamin D analogs are described in U.S. Patent No. 4,851,401 (cyclopentano-Vitamin D analogs), U.S. Patent No. 4,866,048, U.S. Patent No. 5,145,846 (Vitamin D_3 analogs with alkynyl, alkenyl, and alkanyl side chains), U.S. Patent No. 5,120,722 (trihydroxycalciferol), U.S. Patent No. 5,547,947 (fluoro-cholecalciferol compounds), U.S. Patent No. 5,446,035 (methyl substituted Vitamin D), U.S. Patent No. 5,411,949 (23-oxa-derivatives), U.S. Patent No. 5,237,110 (19-Nor-Vitamin D compounds), U.S. Patent No. 4,857,518 (hydroxylated 24-homo-Vitamin D derivatives). Additional Vitamin D analogs are shown in U.S. Patent Nos. 4,804,502; 5,374,629; 5,403,940; 5,446,034; and 5,447,924.

Few attempts have been made to test Vitamin D's antiproliferative effects in humans with cancer. Koeffler et al., *Cancer Treatment Reports* 69:1399-1407, 1985, gave 2 mcg of 1,25-dihydroxyvitamin D daily for 8 weeks or longer to 18 patients with myelodysplastic syndrome. Eight of 18 patients had minor and transient improvements in the peripheral blood counts, but by the end of the 12 week study no patient showed significant improvement and 4 patients experienced symptomatic hypercalcemia. Bower et al., *Lancet* 337:701-702, 1991, treated 19 patients with locally advanced or cutaneous metastatic breast cancer with topical calcipotriol, a Vitamin D analogue.

Three of the 14 patients who completed 6 weeks of treatment showed a 50% reduction in the bidirectional diameter of the treated lesions and one other patient showed minimal response, however hypercalcemia was a complication of the treatment. Palmieri-Sevier et al., *Am. J. Medical Sciences* 306:309-312, 1993, reported a case of long term remission of parathyroid carcinoma which appeared to be induced and maintained by Vitamin D therapy. Rustin et al., *Brit. J. Can.* 74:1479-1481, 1996, performed a clinical trial with a continuous dose of calcitriol in patients with ovarian cancer, and again encountered hypercalcemia.

A phase II trial of oral 1,25-dihydroxyvitamin D (calcitriol) in hormone refractory prostate cancer was reported by Osborn et al., *Urol. Oncol.*, 1:195-198, 1995. Fourteen patients were given a daily oral dose of 0.5-1.5 mcg calcitriol, but no significant response was demonstrated, and clinical deterioration was documented in most of the patients. Thirteen of the patients experienced hypercalcemia, which is the most common side effect of treatment with Vitamin D and its analogs. Concern that hypercalcemic effects of Vitamin D would preclude the achievement of therapeutic, anti-neoplastic serum levels has inhibited the study of the use of this vitamin in humans with cancer. It is an object of this invention to provide a method of treatment with Vitamin D drugs (such as calcitriol) that avoids such hypercalcemia, while permitting the use of this class of drugs in the treatment of tumors and other hyperproliferative diseases.

SUMMARY OF THE INVENTION

Vitamin D and its analogs can be administered in accordance with the present invention, for the treatment of neoplastic diseases, such as the types of tumors mentioned above, which are responsive to treatment with Vitamin D drugs. The method can also be used to treat hyperproliferative skin diseases, such as psoriasis, disorders of keratinization and keratosis, or disorders of sebaceous glands, such as acne or seborrheic dermatitis. The method includes administering to the subject a therapeutically effective pulsed dose of the Vitamin D drug in a sufficient amount to have a therapeutic effect, without inducing hypercalcemia, particularly symptomatic hypercalcemia, for example stage 3 or stage 4 hypercalcemia. This treatment is especially effective to allow the use of highly calcemic drugs (such as drugs having a calcemic index of 0.5 or more) which are often highly effective in the treatment of tumors and hyperproliferative diseases, but which have been avoided in the past because of their calcemic side effects. The dosing regimen of the present invention for the first time allows therapeutically effective antiproliferative (and particularly antineoplastic) amounts of these drugs to be given without inducing the dangerous side effect of life-threatening hypercalcemia, while surprisingly having a prolonged therapeutic specific anti-tumor or general antiproliferative effect.

In a first disclosed embodiment, the Vitamin D drug is administered to a subject having a neoplasm that expresses a Vitamin D receptor, and responds to treatment with a Vitamin D drug. Particular types of tumor that respond to such treatment include adenocarcinomas of the prostate, breast, colon, pancreas and endometrium, as well as small cell and non-small cell cancer of the lung

(including squamous, adenocarcinoma and large cell types), squamous cell carcinoma of the head and neck, transitional cell cancer of the bladder, ovarian and cervical (e.g. squamous cell carcinoma) cancer, renal cell carcinoma, myeloid and lymphocytic leukemia, lymphoma, medullary thyroid carcinoma, melanoma, multiple myeloma, retinoblastoma, and sarcomas of the soft tissues and bone. In particular embodiments, the neoplasm is adenocarcinoma of the breast or prostate.

In yet other specific embodiments, the Vitamin D drug is one that would induce hypercalcemia (particularly symptomatic or life-threatening hypercalcemia) in a subject to which the drug is given at antiproliferative doses. The method would have particular application to drugs that are as calcemic as calcipotriol (calcemic index of about .005-0.01), 11α-fluoromethyl-1α,25-$(OH)_2$-D_3 (having a calcemic index of about 0.1), and drugs having a calcemic index greater than 0.5, for example greater than or equal to 1 (the calcemic index of calcitriol). Drugs with which the method is particularly useful are those drugs having a half-life no greater than about 1 day, for example no greater than about 6 hours, when the dose is given as a therapeutically effective dose. These half-lives are sufficiently short that they allow the blood level to return to non-calcemic levels for a sufficient period between doses so that full osteoclast activation does not occur. In particular embodiments, blood levels of calcium return to normal between doses. The Vitamin D drug is administered in an amount that raises a serum level of Vitamin D in the subject with a tumor to a supraphysiologic amount for a sufficient period of time to induce differentiation or regression of the tumor without causing symptomatic hypercalcemia.

For example, where the Vitamin D analog is calcitriol, it can be administered in a high pulse dose no more than once every three days, for example once a week. Although calcitriol has been used in the past to treat cancer, dosages of such regimens have been 0.5-1.5 mcg per day for prolonged periods of time, which has caused symptomatic hypercalcemia. In accordance with some embodiments of the present invention, calcitriol is orally administered in a dose of at least 0.12 mcg/kg per day (8.4 mcg in a 70 kg person) no more than once every 5 or 6 days, for example once a week. Even higher doses of calcitriol are possible using the pulsed dose regimen of the present invention, for example administering the calcitriol orally in a dose of about at least about 0.48 mcg/kg per day, for example 1 mg/kg per day or higher, e.g. 2-3 mg/kg per day, no more than once per week. As the dosage of the calcitriol or other Vitamin D drug increases, the interval between doses can be increased (for example to as long as 7-10 days) to avoid symptomatic hypercalcemia. It has surprisingly been observed that pulsed increases in the blood level of Vitamin D are sufficient to have an anti-tumor or antiproliferative effect for a prolonged period of time (e.g. 10 days), so that the dosing regimen of the present invention can be followed while encountering a lowered risk of hypercalcemia.

The invention also includes a pharmaceutical composition comprising a Vitamin D drug in a pharmaceutical dosage form containing at least 5 micrograms (mcg) of calcitriol, for example 5-100 mcg. The dosage form may be an oral, intravenous, intramuscular, topical, subcutaneous,

transdermal, sublingual, intranasal, intratumoral or other preparation, but in particular disclosed embodiments the pharmaceutical dosage form is an oral dosage form, such as a tablet or capsule.

The foregoing and other objects, features, and advantages of the invention will become more apparent from the following detailed description of several preferred embodiments.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 is a diagram showing peak and trough plasma calcitriol levels in subjects who received the indicated dose of calcitriol over four hours. Peak levels (◊) were determined at 6 hours after administration, and trough levels (O) were determined at 48 hours after administration.

FIG. 2 shows a time course of plasma calcitriol levels in a subject who received a 2.0 μg/kg dose of calcitriol.

DETAILED DESCRIPTION OF SEVERAL EMBODIMENTS

Definitions

The following definitions will help with an understanding of the terms used in this specification.

A "Vitamin D drug" is a drug that raises the blood or tissue level of Vitamin D, or has an affinity for the Vitamin D receptor, for example binding to that receptor with a Relative Competitive Index (RCI) of 0.05 or greater, more particularly 5 or greater, for example 5-250. The RCI is indexed to an RCI of 100 for calcitriol. The term also includes any of the family of secosteroids with antirhichitic activity, such as Vitamin D_2 (ergocalciferol) and Vitamin D_3 (cholecalciferol), their precursor molecules such as ergosterol (7-dehydro-22-dehydro-24-methyl-cholesterol) and 7 dehydrocholesterol, 25-hydroxyvitamin D_3, the 3-hydroxylated dihydrotachysterol$_2$, the 1α-hydroxylated alfacalcidol (1α-hydroxyvitamin D_3) and calcitriol (1α, 25-dihydroxyvitamin D_3), as well as the numerous natural and synthetic Vitamin D analogs set forth in the attached Appendix 1 (from Bouillon et.al, *Endocrine Reviews* 16: 200-257,1995).

Vitamin D drugs also include Vitamin D preparations and analogs that are currently in clinical use, such as Rocaltrol® (Roche Laboratories), Calcijex® injectable calcitriol, investigational drugs from Leo Pharmaceutical including EB 1089 (24a,26a,27a-trihomo-22,24-diene-1αa,25-$(OH)_2$-D_3), KH 1060 (20-epi-22-oxa-24a,26a,27a-trihomo-1α,25-$(OH)_2$-D_3), MC 1288 and MC 903 (calcipotriol), Roche Pharmaceutical drugs that include 1,25-$(OH)_2$-16-ene-D_3, 1,25-$(OH)_2$-16-ene-23-yne-D_3, and 25-$(OH)_2$-16-ene-23-yne-D_3, Chugai Pharmaceuticals 22-oxacalcitriol (22-oxa-1α,25-(OH)2-D_3; 1α-(OH)D_5 from the University of Illinois; and drugs from the Institute of Medical Chemistry-Schering AG that include ZK 161422 and ZK 157202. Appendix 3 provides additional information about chemical structure, route of administration and dosing of some of these compounds. Vitamin D analogs also include topical preparations of Vitamin D, such as Calcipotriene (Dovonex) and Tacalcitol, used in the treatment of psoriasis.

A "Vitamin D receptor" (or VDR) is a protein transcription factor, for which the gene and its product have already been characterized and found to contain 427 amino acids, with a molecular weight of about 47,000, or variants thereof. The full length cDNA of the human VDR is disclosed in Baker et al., *PNAS, USA* 85:3294-3298, 1988.

"Tumor cells that express (or contain) the Vitamin D receptor" are those tumors that have been shown to contain the Vitamin D receptor, tumors that are subsequently shown to contain the receptor (using immunohistochemical or other techniques), tumor types (such as breast cancer) that have demonstrated a clinical improvement in response to treatment with calcitriol or its analogs or other Vitamin D drugs, and tumors for which there is epidemiologic data demonstrating an association between low Vitamin D levels and higher cancer incidence (such as adenocarcinomas of the prostate, breast and colorectum). The presence of Vitamin D receptors can be determined by any means known in the art, such as any of the techniques disclosed in Pike, *Ann. Rev. Nut.* 11:189-216, 1991.

"Elevated (or supraphysiologic) level of Vitamin D" refers to a 1,25-dihydroxyvitamin D plasma concentration greater than about 0.15 nm (65 pg/ml), or other Vitamin D concentration greater than normal in the laboratory where the concentration is measured, for example in humans a total human plasma concentration greater than about 10 ng/ml of 25-hydroxyvitamin D (although this and all other normal values can vary depending on the techniques used to measure the concentration).

"Hypercalcemia" refers to a calcium plasma concentration greater than normal in the laboratory where the concentration is measured, for example greater than about 10.5 mg/dL in humans (although this and all other normal values can vary depending on the techniques used to measure the concentration). Hypercalcemia can be broken into grades 0-4, as set forth in Appendix II.

"Symptomatic hypercalcemia" refers to laboratory demonstrated hypercalcemia associated with one of more of the signs or symptoms of hypercalcemia. Early manifestations of hypercalcemia include weakness, headache, somnolence, nausea, vomiting, dry mouth, constipation, muscle pain, bone pain, or metallic taste. Late manifestations include polydypsia, polyuria, weight loss, pancreatitis, photophobia, pruritis, renal dysfunction, aminotransferase elevation, hypertension, cardiac arrhythmias, psychosis, stupor, or coma. Ectopic calcification has been reported when the calcium-phosphate product (multiplying the concentrations of calcium and phosphate) exceeds 70. "Severe symptomatic hypercalcemia" refers to grade 3 or grade 4 hypercalcemia.

A "tumor" is a neoplasm, and includes both solid and non-solid tumors (such as hematologic malignancies). A "hyperproliferative disease" is a disorder characterized by abnormal proliferation of cells, and generically includes skin disorders such as psoriasis as well as benign and malignant tumors of all organ systems. "Differentiation" refers to the process by which cells become more specialized to perform biological functions, and differentiation is a property that is totally or partially lost by cells that have undergone malignant transformation.

A "therapeutically effective dose" is a dose which in susceptible subjects is sufficient to prevent advancement, or to cause regression of the disease, or which is capable of relieving symptoms

caused by the disease, such as fever, pain, decreased appetite or chachexia associated with malignancy.

A "pulse" dose of a Vitamin D drug refers to administration of the drug in a sufficient amount to increase the blood or tissue level of Vitamin D to a supraphysiologic concentration for a sufficient period of time to have a therapeutic benefit, but with a sufficient period between doses to avoid hypercalcemia, given the pharmacological half life of the drug, its rate of elimination from the body, and its calcemic index.

The "calcemic index" of a drug is a measure of the relative ability of a drug to generate a calcemic response, for example as measured and reported in Bouillon et al., *Endocrine Reviews* 16:200-257, 1995. A calcemic index of 1 corresponds to the relative calcemic activity of calcitriol. A calcemic index of about 0.01 corresponds to the calcemic activity of calcipotriol. A calcemic index of 0.5 would correspond to a drug having approximately half the calcemic activity of calcitriol. The calcemic index of a drug can vary depending on the assay conducted, e.g. whether measuring stimulation of intestinal calcium absorption (ICA) or bone calcium mobilizing activity (BCM), as reported in Hurwitz et al., *J. Nutr* 91:319-323, 1967 and Yamada et al., Molecular, *Cellular and Clinical Endocrinology* (Berlin), pages 767-774, 1988. Hence relative calcemic activity is best expressed in relation to the calcemic activity of calcitriol, which is one of the best characterized Vitamin D drugs.

Vitamin D Drugs

Normal serum levels of 1,25-dihydroxyvitamin D range between 0.05 and 0.16 nM, however therapeutic drug levels necessary for cancer inhibition have not been well defined. Skowronski et al. (*Endocrinology* 136-20-26, 1995) demonstrated measurable growth inhibition of LNCaP human prostate cancer cells in vitro at a 1,25-dihydroxyvitamin D concentration of 0.1 nM and 50% growth inhibition at a 1.0 nM concentration. Peehl et al. (*Cancer Research* 54:805-810, 1994) incubated human prostate cancer cells in primary culture with 1,25-dihydroxyvitamin D concentrations ranging between 0.025 and 25 nM and demonstrated half maximal growth inhibition at levels between 0.25 and 1.0 nM. Previous clinical trials of Vitamin D in the treatment of cancer have proceeded on the assumption that high levels of the drug were needed for a prolonged period of time to have a therapeutic benefit. The inventors of the present invention, however, have surprisingly shown that intermittent supraphysiologic levels of 1,25-dihydroxyvitamin D (for example greater than or equal to 0.25 nM) are sufficient to inhibit cancer growth and other proliferative disease in mammals. This surprising finding now permits the therapeutic benefits of Vitamin D therapy to be achieved without substantial risk of morbidity from iatrogenic hypercalcemia induced by the therapy.

Calcitriol is a short acting preparation of 1,25-dihydroxyvitamin D, which therefore offers an opportunity for intermittent treatment aimed at achieving high serum 1,25-dihydroxyvitamin D levels for brief periods of time. This regimen has surprising anti-tumor activity, while minimizing toxicity, such as hypercalcemia. Calcitriol has primarily been studied when chronically administered

as replacement therapy, for which its usual dose is 0.25-1.0 mcg per day. Peak serum concentration is reached at 2 hours and serum half life is 3-6 hours. Intestinal absorption of calcium begins to increase 2 hours after administration. Hypercalcemic effect is maximal at 10 hours and lasts 3-5 days.

In one embodiment of the invention, a sufficient dose of calcitriol is administered to raise serum 1,25-hydroxyvitamin D levels to a therapeutically effective level for a pulsed dose that has an anti-proliferative effect without causing significant hypercalcemia (for example symptomatic grade 3 or grade 4 hypercalcemia). With calcitriol, an example of such a dose would produce a serum level of at least about 0.5 nM, for example about 0.9 nM or more (e.g. 1-25 nM, for example 5-10 nM), for at least 2 hours (e.g. 2-5 hours) and preferably no more than 6 hours. In particular embodiments, the pulsed dose of calcitriol does not exceed a dose at which symptomatic hypercalcemia occurs, or more preferably a pulsed dose at which even laboratory hypercalcemia occurs.

Information about short term effects of higher than replacement doses of calcitriol is available for helping predict drug effects. Papapoulus et al., *(Clinical Science* 62:427-429, 1982) gave 2 mcg of calcitriol as a single oral dose to healthy volunteers and achieved peak 1,25-dihydroxyvitamin D serum concentrations of 0.235 and 0.351 nM. Mason et al. (*BMJ* 1980:449-450) gave a single oral dose of 4 mcg calcitriol to healthy volunteers and achieved peak 1,25-dihydroxyvitamin D serum concentrations of 0.42 nM with no elevation in serum calcium. Brickman et al. (*Am. J. Med.* 57:28-33, 1974) treated normal volunteers with calcitriol doses up to 2.7 mcg/day for 7 to 15 days. While calcium absorption and excretion were increased, no significant elevations in serum calcium were observed. Adams et al. (*Kidney Int.* 21:90-97, 1982) treated normal volunteers with up to 3 mcg/day of calcitriol for 6-12 days and achieved stable 1,25-dihydroxyvitamin D serum levels of 0.184-0.235 nM. None of the patients who were on a low calcium diet experienced elevation in serum calcium. Geusens et al. (*Calcified Tissue Int.* 49:168-173, 1991) gave 4 mcg of calcitriol per day for 4 days to 27 postmenapausal women with osteoporosis or osteoarthritis. They demonstrated increased urinary calcium excretion but no increase in urinary hydroxyproline excretion. Four of the 27 patients had a serum calcium above 10.8 but no clinically significant hypercalcemia was observed.

Antiproliferative levels of 1,25-dihydroxyvitamin D can be achieved for short periods of time with minimal adverse effects, particularly if hypercalcemia during short course 1,25-dihydroxyvitamin D therapy is primarily mediated by increases in intestinal calcium absorption (slower calcium elevation) rather then osteoclast activation (which can rapidly mobilize calcium from bone). Higher 1,25-dihydroxyvitamin D levels are achievable when the drug is given in conjunction with a reduced calcium diet to minimize intestinal calcium absorption, and adequate hydration to maximize calcium excretion. The maximal tolerated dose of calcitriol, when given intermittently has not been defined, but doses as high as 0.48 mcg/kg have been tolerated without hypercalcemia.

Higher doses of a Vitamin D drug, sufficient to achieve therapeutic antiproliferative levels, may also be achieved by administering the drug in conjunction with bisphosphonate osteoclast inhibitors, such as pamidronate. Selby et al. (*Endocrinology* 108:1083-1086, 1981) provided an

example of treating hypercalcemia due to Vitamin D with pamidronate. The potential for achieving high serum 1,25-dihydroxyvitamin D levels when osteoclasts are inhibited in patients with osteopetrosis is possible with calcitriol doses as high as 32 mcg/day for 3 months (Key et al., *NEJM* 310:409-415, 1984) where stable serum levels of 1,25-dihydroxyvitamin D peaked at 2.32 nM with no hypercalcemia.

The following Examples illustrate the general method of the present invention, as well as specific case histories to illustrate its use. These Examples also provide a general framework for evaluating other Vitamin D drugs, and determining a therapeutically effective dose of a Vitamin D drug in a subject with a Vitamin D responsive hyperproliferative disease, without inducing symptomatic iatrogenic hypercalcemia.

EXAMPLE 1

General Treatment Plan

A patient with a known Vitamin D receptor positive tumor (such as adenocarcinoma of the prostate, breast, lung, colon or pancreas, or transitional cell carcinoma of the bladder, or melanoma) may be placed on a prescribed reduced calcium diet prior to treatment, to help minimize intestinal absorption and allow even higher doses of the Vitamin D drug to be used. This reduced calcium diet may be continued for the duration of treatment, and for one week after the last dose of the Vitamin D drug. The diet ideally restricts daily calcium intake to 400-500 mg, by avoiding all dairy products, as well as sardines and other fish canned with their bones, legumes, greens, and any calcium fortified foods or drinks. The subject is then asked to drink 4 - 6 cups of fluid more than usual intake starting 12 hours before treatment and continuing for days 1, 2, and 3, to assure adequate oral hydration. Magnesium containing antacids, oral calcium supplements, cholestyramine, colestipol, and other bile resin binding agents may also be avoided during treatment.

Baseline laboratory tests that may be obtained include serum levels of calcium, phosphate, and 1,25-dihydroxyvitamin D. At the initial dose level, e.g. calcitriol 0.06 mcg/kg po (or another Vitamin D drug for which the dose is to be determined) is divided into 4 doses, and one of those four doses is taken during each hour for 4 hours until the total 0.06 mcg/kg dose is taken. Alternatively, a single higher dose formulation may be ingested. The doses may be rounded up to the nearest 0.5 mcg. The subject is monitored daily for symptoms of hypercalcemia for at least 2-3 days following administration.

The patient may have a variety of laboratory tests performed to monitor the presence of hypercalcemia, or any physiological consequences of hypercalcemia. Such tests may include calcium at 0, 24, 48 hours, and baseline levels of creatinine, total billirubin, ALT, alkaline phosphatase, and a complete blood count. Other possible laboratory tests include phosphate, 1,25-dihydroxyvitamin D levels at 0, 6, 24, 48 hours, and 24 hour urine collection for calcium and hydroxyproline on day 2. Subjects are treated with the once a week pulse dose of Vitamin D weekly until disease progression or

4 weeks, whichever comes first, and are followed for 2 months from enrollment. If Grade 3 toxicity is encountered, the treatment is stopped.

An initial dose may be chosen from safe doses documented in the literature, followed by a multistage escalation scheme, such as the one described by Gordon and Willson (*Statistic in Medicine* 11:2063-2075, 1992). Patient accrual occurs in stages I, II, and III. The stages require the accrual of one, three, or six patients respectively before dose escalation. All patients enrolled at a dose level complete 4 weeks of treatment before the dose level is escalated. In stage I, a single patient is entered at each dose level. Accrual continues in stage I until the first Grade 3 toxicity is encountered. When a Grade 3 toxicity is encountered, two more patients are accrued at the same dose level and accrual will continue in stage II. Doses are reduced one level if one Grade 4 or 5 toxicity is encountered in stage I.

Accrual continues in stage II if no Grade 3 toxicities are encountered. When one or two Grade 3 or Grade 4 toxicities are encountered, three additional patients are accrued at the same dose level and accrual continues in stage III. Doses are reduced one level if one Grade 5 or three Grade 3 or Grade 4 toxicities are encountered in stage II. In stage III six patients are enrolled at each dose level. If only one Grade 3 toxicity is encountered, the dose will be escalated and the accrual will revert to stage II. If two or more Grade 3 or greater toxicities occur, no further escalation will occur. The MTD (maximum tolerated dose) is defined as that dose at which 1/3 or fewer of the subjects experience grade 3 toxicity. For calcitriol, the initial dose was 0.06 mcg/kg po over 4 hours. At each successive level, the dose is doubled until the first grade 3 toxicity is encountered. After that, each dose increase is 1.33x of the preceding level according to a modified Fibonacci scheme (Dillman and Koziol, *Molecular Biotherapy* 4:117-121, 1992).

For calcitriol, the pulse dose was given to each subject weekly, and the subject was monitored for early signs and symptoms of hypercalcemia, such as weakness, headache, somnolence, nausea, vomiting, dry mouth, constipation, muscle pain, bone pain, metallic taste. The patient was also monitored for any more serious manifestations, such as polydypsia, polyuria, weight loss, pancreatitis, photophobia, pruritis, renal dysfunction, aminotransferase elevation, hypertension, cardiac arrhythmias, psychosis, stupor, coma, and ectopic calcification. Appropriate treatment is instituted for any patient who demonstrates hypercalcemic toxicity, and the calcitriol is stopped until serum calcium returns to normal.

The following Table I illustrates a protocol that can be followed with each drug to determine a tolerated pulse dose. A protocol for determining a therapeutic dose will be described in Example 2.

TABLE 1

Example of Protocol for Determining Tolerated Dose

Evaluation & Procedures	EVALUATION	WEEKS 1-4			WEEKS 5-8			
	Screen	Day 1	Day 2	Day 3	Follow-Up		Premature	
					Week 5	Week 7	Until Ca nl daily	After Ca nl every 2
Informed Consent	x							
Inclusion / Exclusion Criteria	x							
Physical Exam	x	x				x	x	
Sitting Vital Signs	x	x					x	
Adverse Effects Recorded			x	x	x	x	x	x
Calcitriol administered		x						
Calcium	x	x	x	x	x	x	x	x
Phosphate	x	x	x	x			x	
1,25-dihydroxyvitamin D level	x	xx	x	x			x	
Creatinine	x	x					x	
Total Billirubin	x	x					x	
ALT	x	x					x	
Alkaline Phosphatase	x	x					x	
Albumin	x							
Complete Blood Count	x	x						
β-hCG (select patients)	x							
Urine Collection	x		x					
Diet Questionnaire		x						
Tumor measurements (when appropriate)	x					x		

EXAMPLE 2

Determination of Therapeutically Effective Dose

Tumor markers, such as PSA, CA 15-3, and others can be used to assess tumor progression or regression, although the results of such assays can sometimes be difficult to interpret because administration of Vitamin D has been shown to increase tumor marker production while inhibiting cancer cell growth. This effect is presumably due to the differentiation inducing properties of Vitamin D.

Alternative means for determining a therapeutic response can also be employed, for example direct radiographic measurement of tumor lesions. A measurable lesion may be considered one that is bidimensionally measurable, with clearly defined margins on physical exams, x-ray, or scan. At least one diameter is preferably greater than 0.5 cm. Bone lesions are not included.

Evaluable disease includes unidimensionally measurable lesions, masses with margins not clearly defined, palpable nodal disease, lesions with both diameters less than 0.5 cm, and bone disease. Non-evaluable disease includes disease manifested by pleural effusions, ascites, or disease

documented by indirect evidence only (e.g., by lab values which fall into a category of not being evaluable). The objective status is recorded at entry into the trial and during week 7 (where week 1 is the week during which the first dose of the Vitamin D drug is given). If an organ has too many measurable lesions to measure at each evaluation, a specific number (such as three lesions) are selected to be followed before the patient is entered in the study.

A complete response (CR) is the complete disappearance of all measurable and evaluable disease, with no new lesions. If the subject has effusions, ascites or disease assessable by surgical restaging (e.g., testicular and extragonadal gem cell cancer), the disease must be cytologically negative. Patients with markers or indirect evidence of involvement must have normalization of abnormal values. All measurable, evaluable and non-evaluable lesions and sites must be assessed. A partial response (PR) is found in subjects with at least one measurable lesion with □50% decrease of perpendicular diameters of all measurable lesions, with no progression of evaluable disease, and no new lesions. All measurable and evaluable lesions and sites must be assessed. In lung cancer, a greater than 50% decrease in estimated area of evaluable, but non-measurable, tumor mass, as agreed upon by two independent observers, not to include pleural effusions. Stabilization is a response that does not qualify as a complete response, partial response or progression.

EXAMPLE 3

Treatment of Breast Cancer

In this example, a 42 year old woman with breast carcinoma metastatic to numerous sites in the skeleton received a dose of 11 mcg of calcitriol (Rocaltrol, Roche) administered as 22 tablets (0.5 mcg each tablet) divided into four nearly equal doses given in hour one, two, three and four. The patient received this same therapy on day 1, 8, 15 and 22, and then was observed on study until day 56, and tolerated the treatment well. She had no Grade II or higher toxicities on the NCI toxicity grading scale (Appendix 2). Subjective beneficial effects observed included a reduction in pain and in analgesia required. Objective benefits included a progressive decrease in the serum tumor marker CA15-3 from 445 (pre-treatment) to 365 (day 27), 365 (day 48) and 320 (day 70). Radiologic evaluation of areas of known bony involvement showed progressive sclerosis of multiple lesions in the pelvis and right hip, indicating bone healing as a positive response to therapy. No new lesions or pathologic fractures identified were identified by day 64.

EXAMPLE 4

Treatment of Melanoma

In this example a 72 year old man with metastatic malignant melanoma of the right jaw received a dose of 37 mcg of calcitriol (Rocaltrol, Roche) administered as 74 tablets (0.5 mcg each tablet) divided into four nearly equal doses given in hour one, two, three and four. The patient received this same therapy on day 1, 8, 15 and 22, and observed until at least day 56. The level of calcitriol in a plasma sample obtained two hours after the last dose of calcitriol (on week one) was

determined using a commercial assay at Endocrine Sciences, Inc. The level was 1826 pg/ml, compared to the range for calcitriol levels in normal controls being 21 to 65 pg/ml. In spite of the markedly elevated levels of calcitriol achieved with this weekly schedule, this patient did not have any subjective or objective toxicity. Levels of serum calcium and other chemical and hematological parameters in the blood remained normal.

EXAMPLE 5

Summary of Trial Results

Patients

Eligibility criteria included histologically confirmed malignancy refractory to standard therapy; age $\geq$ 18 years; expected survival of > 2 months; ECOG performance status $\leq$ 2; hematocrit $\geq$ 30; serum creatinine $\leq$ 1.2 mg/dL; serum calcium $\leq$ 10.5 mg/dL; serum phosphate $\leq$ 4.2 mg/dL; ALT $\leq$ 60 IU/L; total serum bilirubin < 2 mg/dL. Exclusion criteria included pregnancy, history of hypercalcemia, kidney stones, heart failure or significant heart disease including myocardial infarction in the last 3 months, known cardiac ejection fraction less than 30%, current digoxin therapy, thiazide diuretic therapy within 7 days, bisphosphonate treatment within 4 weeks, systemic steroid therapy within 2 weeks, and unwillingness or inability to stop all magnesium containing antacids, bile resin binding drugs, or calcium supplements for the duration of the study.

Treatment

Baseline evaluation included a complete history and physical exam, complete blood count, serum creatinine, serum calcium, serum phosphate, total serum bilirubin, ALT, alkaline phosphatase, albumin, serum β-hCG in women of childbearing potential, 24 hour urine collection for calcium, and tumor measurements.

Patients were asked to maintain a reduced calcium diet for the four treatment weeks, with a goal of less than 500 mg per day, as described in Example 1. Calcitriol (Rocaltrol®, Roche Pharmaceuticals) was given orally once a week for four weeks. Each weekly dose was given in four divided doses given hourly over four hours. The starting dose was 0.06 μg/kg.

Monitoring

Complete blood count, serum creatinine, total serum bilirubin, ALT, alkaline phosphatase were monitored weekly. Serum calcium and phosphate were checked on the treatment day (day 1), and on days 2 and 3. Twenty-four hour urinary calcium excretion was measured on day 2. The 1,25-dihydroxyvitamin D levels were measured by ^{125}I radioimmunoassay (Incstar, Stillwater, MN) and by a radioreceptor assay using calf thymus 1,25-dihydroxyvitamin D receptor (Endocrine Sciences, Calabasas Hills, CA). Peak levels were measured two hours after all the pills had been ingested. Trough levels were measured approximately 48 hours later.

Compliance with the diet was monitored with a seven day dietary recall questionnaire

directed at calcium rich foods. Daily calcium intake was estimated by adding the calcium content of calcium rich foods identified by the questionnaire to the estimated calcium content of the basal diet. The calcium content of the basal diet was calculated to be 1 mg of calcium/8 Kcal. Caloric intake was estimated with the use of the Food Processor 7.0 software (ESHA Research, Salem, OR).

After completing the four week treatment period, patients were monitored for four additional weeks. Serum calcium was checked in weeks 5 and 7 and tumor measurements were obtained in week 7. All toxicities were graded according to NCI Common Toxicity Criteria. Response was assessed according to WHO guidelines.

Statistical considerations

The planned dose escalation was governed by the multistage escalation scheme described by Gordon and Willson, 1992. The maximal tolerated dose (MTD) was defined as that dose at which 1/3 or fewer of the patients experienced Grade 3 toxicity (64). Patients who had evidence of response or stable disease, and no Grade 3 or greater toxicity were permitted to reenroll and receive either the same dose or the next higher dose of calcitriol. Statistical analysis was performed using StatView 5.0 for Windows software (SAS Institute, Cary, NC)

RESULTS

Fifteen different patients were enrolled in 20 cycles of therapy (Table 2). Two patients were withdrawn from the study prior to completion of the four week regimen because of disease progression. No patient withdrew because of toxicity of therapy or unacceptability of the diet. Five patients reenrolled for a second cycle of treatment.

Table 2
Individual Patients Enrolled on Study

Patient	Age	Gender	Malignancy	Cycle 1 dose (μg/kg)	Cycle 2 dose (μg/kg)
1	79	male	Adenocarcinoma of the prostate	0.06	0.12
2	42	female	Adenocarcinoma of the breast	0.12	
3	70	male	Adenocarcinoma of the lung	0.24	
4	72	male	Melanoma	0.48	
5	53	male	Squamous Cell of the tonsil	0.48	
6	48	female	Hepatocellular carcinoma	0.80	1.60
7	80	male	Adenocarcinoma of the prostate	0.96	2.00
8	53	female	Adenocarcinoma of the breast	1.60	
9	77	female	Adenocarcinoma of the lung	1.92	2.00
10	78	male	Adenocarcinoma of the prostate	2.00	
11	69	male	Adenocarcinoma of the prostate	2.00	
12	46	female	Adenocarcinoma of the breast	2.00	
13	47	female	Gastrointestinal stromal tumor	2.00	
14	71	male	Adenocarcinoma of the pancreas	2.80	2.80
15	76	male	Adenocarcinoma of the prostate	2.80	

No deaths occurred. No patient withdrew from the study due to toxicity, and no Grade 3 or higher toxicity was seen. All observed toxicities are listed in Table 3.

Table 3
Toxicities developed during each treatment course (N = 20)

Toxicity	Grade 1	Grade 2	Grade 3	Grade 4
Leukopenia	5[1]	1[2]	0	0
Anemia	3	4[2]	0	0
Thrombocytopenia	2	0	0	0
Hypercalcemia	8	0	0	0
Creatinine elevation	4	0	0	0
Bilirubin elevation	2	0	0	0
ALT elevation	1	0	0	0
Alkaline phosphatase elevation	2	1[2]	0	0
Nausea and vomiting	5	2	0	0
Diarrhea	3	1	0	0
Constipation	5	0	0	0
Dyspepsia	4	0	0	0
Headache	5	0	0	0
Fever	2	0	0	0
Skin rash	1	0	0	0
Bone or muscle pain	8	0	0	0

[1]All were within normal limits of our laboratory (3.4 – 10.0 k/mm^3) but fell into the Grade 1 toxicity range of 3.0 – 3.9 k/mm^3
[2]All had Grade 1 abnormalities prior to entry onto study.

The normal range for serum 1,25-dihydroxyvitamin D levels is 0.05 – 0.16 *nM* (20 – 65 pg/ml). An approximately linear increase in the peak level was observed with increasing dose until the 0.48 μg/kg dose (Table 4, Figure 1). Above this dose, a further elevation of peak levels was not seen. Serum 1,25-dihydroxyvitamin D trough levels returned to normal or near normal levels by 48 hours (Figure 1). A limited study of calcitriol pharmacokinetics showed the expected decay in 1,25-dihydroxyvitamin D levels after hour 6 (Figure 2).

Table 4
Mean Peak and 48 hour 1,25-dihydroxyvitamin D levels by dose

Dose level (μg/kg)	Patients	Mean peak (nM)	Mean 48 hour level
0.06	1	0.71	0.27
0.12	2	1.10	0.14
0.24	1	2.27	0.21
0.48	2	4.11	0.23
0.80	1	3.53	
0.96	1	3.83	
1.60	2	3.65	
1.92	1	3.34	
2.00	6	4.07	0.26
2.80	2	2.96	

Mean serum calcium (normal range 8.5 – 10.5 mg/dL) increased from 9.55 (SD 0.57) mg /dL prior to treatment to 9.76 (SD 0.63) mg/dL 24 hours later and to 9.88 (SD 0.68) mg/dL at 48

hours ($p=0.0002$ by a two way repeated measures analysis of variance). All calcium levels above the normal range returned to normal within 2 days with no intervention. Mean serum phosphate (normal range 2.2 – 4.2 mg/dL) increased from 3.43 (SD 0.56) mg /dL prior to treatment to 3.98 (SD 0.57) mg/dL 24 hours later and dropped to 3.86 (SD 0.53) mg/dL at 48 hours ($p<0.0001$ by a two way repeated measures analysis of variance). Mean 24 hour urinary calcium excretion (normal range 100 – 300 mg) increased from 130 (SD 62) mg with a range of 44 – 292 mg prior to treatment to 263 (SD 125) mg with a range of 59 – 594 on treatment, measured on day 2 of each treatment week ($p<0.0001$ by a one way repeated measures analysis of variance). There was no statistically significant increase in urinary calcium excretion during the treatment period by the Bonferroni/Dunn test.

Five of eight patients with measurable disease had stable disease. Among them, an adenocarcinoma of the lung patient, an adenocarcinoma of the pancreas patient, and a hepatocellular carcinoma patient received two cycles of therapy and remained stable for the entire 16 weeks of their time on study. The hepatocellular carcinoma patient had an associated 70% decline in her serum AFP level. The remaining three patients with measurable disease had evidence of progressive disease.

Four of seven patients without measurable disease had no evidence of progression. Among them was the breast cancer patient described in Example 3. A prostate cancer patient received two cycles of therapy, and had a stable PSA for the entire 16 weeks during which the drug was administered, in spite of a rapidly rising PSA prior to enrollment. The remaining three patients without measurable disease had either tumor marker or clinical evidence of progressive disease.

No patient developed dose-limiting hypercalcemic toxicity from calcitriol (< 2 mcg/day). Measurements of peak blood calcitriol levels in patients indicate that blood levels (up to 8.9 nM) are at a level known to be growth inhibitory for cancer cells in culture. Furthermore, the drug calcitriol is essentially completely cleared from the blood by day 3, and this rapid clearance explains the increased safety profile of the weekly pulse schedule.

An unanticipated result was the finding that escalation of calcitriol dose beyond dose level 0.48 μg/kg did not result in further increases in peak calcitriol levels. More detailed measurement of calcitriol levels in one patient (dose level 2.0 μg/kg) indicated that absorption is saturated at high doses rather than delayed, as neither the peak levels of calcitriol are delayed and the half-life of the drug is not extended beyond the usual time observed in lower dose studies. The maximal tolerated dose (MTD) of calcitriol was not determined by the data presented in this example.

In summary, pulsed weekly administration of calcitriol allows substantial escalation of the total weekly dose of calcitriol that can be administered to patients with advanced malignancies. Peak blood levels of calcitriol about 25 fold above the upper limit of normal are achieved with minimal toxicity. These levels are well into the range where antiproliferative effects of calcitriol are observed. Based on the observation that blood levels of calcitriol do not increase linearly with increased dose beyond the 0.48 μg/kg level, a dose level of 0.5 μg/kg is an example of a dose that is therapeutically effective in patients whose tumor responds to this therapy, but which does not result in unacceptable hypercalcemia.

EXAMPLE 6

Preparation of Pharmaceutical Dosage Forms

Preparation of pharmaceutically acceptable compositions of the Vitamin D drugs of the present invention can be accomplished using methods well known to those with skill in the art. Any of the common carriers such as sterile saline solution, plasma, etc., can be utilized with the Vitamin D drugs of the invention. Routes of administration include but are not limited to oral, intracranial ventricular (icv), intrathecal (it), intravenous (iv), parenteral, rectal, topical ophthalmic, subconjunctival, nasal, aural and transdermal. The Vitamin D drugs of the invention may be administered intravenously in any conventional medium for intravenous injection such as an aqueous saline medium. Such medium may also contain conventional pharmaceutical adjunct materials such as, for example, pharmaceutically acceptable salts to adjust the osmotic pressure, buffers, preservatives and the like. Among such media are polysorbate, normal saline, lactated Ringer's solution, and plasma. Vitamin D is somewhat insoluble, hence solubilizing agents such as sesame oil, or equivalent lipophilic preparations, may be used to administer the Vitamin D drug.

Embodiments of the invention comprising medicaments, such as tablets or capsules, can be prepared with conventional pharmaceutically acceptable carriers, adjuvants and counterions as would be known to those of skill in the art. The medicaments are preferably in the form of a unit dose in solid, semi-solid and liquid dosage forms such as tablets, pills, powders, liquid solutions or suspensions, and injectable and infusible solutions, for example a unit dose vial. Effective dosage ranges included in the unit dose for calcitriol vary from about 5 mcg to about 100 mcg. The unit dosages of the clacitriol are much higher than previously used, because of the unanticipated finding that high pulse, therapeutically effective doses of the drug can be given without inducing symptomatic hypercalcemia.

EXAMPLE 7

Determining Binding Affinity

Binding affinity of the Vitamin D drugs for the Vitamin D receptor can be determined by any acceptable means, such as the VDR binding analysis and Scatchard plots in Peehl et al., *Cancer Research* 54:805-810, 1994., which is incorporated by reference.

VDR affinity can be assayed by a competitive receptor assay with radio-labeled calcitriol to determine the Relative Competitive Index (RCI) wherein the RCI for calcitriol is set at 100. The RCI of some of the Vitamin D analogs is set forth in Appendix 3.

EXAMPLE 8

Detecting Vitamin D Receptor on Tumor Cells

The presence of the VDR on tumor cells can be detected by the methods set forth in Peehl et al., which has been incorporated by reference in Example 7. A variety of other assays can be used to detect the VDR, including immunohistochemistry (Kaiser et al., *J. Cancer Res. Clin. Onc.* 122:356-

359, 1996); Western blot (Cross et al., *Anticancer Research* 16:2333-2338, 1996); ligand binding assays and DNA probe hybridization to RNA (Northern blot)(*Endocrinology* 132:1952-1960); and detection of RNA by ribonuclease protection assay (Shabahang et al., *Annals of Surg. Onc.* 3:144-149, 1996).

In view of the many possible embodiments to which the principles of our invention may be applied, it should be recognized that the illustrated embodiment is only a preferred example of the invention and should not be taken as a limitation on the scope of the invention. Rather, the scope of the invention is defined by the following claims. We therefore claim as our invention all that comes within the scope and spirit of these claims.

Summary of biological data for analogs of $1\alpha,25(OH)_2D_3$

APPENDIX I

$1\alpha,25$-$(OH)_2$-VITAMIN D_3 ANALOG NAME	ANA-LOG	VIT D RECEPTOR (non Human)			VIT D RECEPTOR (HL-60)		D-BINDING PROTEIN		CELL DIFFERENTIATION			CALCEMIC INDEX		
	CODE NAME	RCI	SPC	(ref)	RCI	(ref)	RCI	(ref)	ED50	(s)	(ref)	CI	(s)	(ref)
$1\alpha,25$-$(OH)_2$-26,27-d_6-D_3	A	0.85 100	c r	(144) (227,186)					1.00	h	(186)	0.92,0.64	c	(227,186)
$1\alpha,25$-$(OH)_2$-22-ene-D_3	B	86 122	c r	(144) (186)	79	(143)	165 150	(143) (143)	1.25	h	(186)	0.9,1.3	c,i	(186)
$1\alpha,25$-$(OH)_2$-D_3	C	100	c	(144)	100	(143)	100	(143)	1.00			1		
$1\alpha,25$-$(OH)_2$-26,27-F_6-22-ene-D_3	D	21 60	c r	(144) (186)					30.00	h	(186)	2.2,1.1	c,i	(186)
$1\alpha,25$-$(OH)_2$-26,27-F_6-D_3	E	15 90 30 <100 80	c c c c r	(144) (220) (499,223) (226) (186)	12	(144)	32 40 <100 40	(143) (220) (499) (143)	10.00 20.00	h h	(223,558) (186)	10 2.3,1.4	sc c,i	(499) (186)
$1\alpha,25S$-$(OH)_2$-26-F_3-D_3	F	46	c	(144)	25	(143)								
$1\alpha,25$-$(OH)_2$-24-F_2-D_3	G	79 230 100	c r c	(144) (186) (215,502)	50 100	(143) (211)			7.00 20.00 2.00 10.00	h h u h	(211) (212) (212) (186)	1 10 10 4 2.35,0.6 >1 4 5	c,i c sc sc c,i r cbp sc	(494) (214) (214) (209) (186) (215) (491,497) (216)
$1\alpha,25S,26$-$(OH)_3$-22-ene-D_3	H	34 5	c c	(144) (557)	39	(143)	9	(143)	1.00	h	(557)			
$1\alpha,25R,26$-$(OH)_3$-22-ene-D_3	I	29	c	(144)	50	(143)								
$1\alpha,25$-$(OH)_2$-D_2	J	61 100 100	c c c,p,r	(144) (198,509,513) (550)	64	(143)	370	(143)	0.50	h	(193)	1 1	sc c,i	(513) (550)
$1\alpha,25$-$(OH)_2$-24-epi-D_2	K	24 50 30 20	c c c c	(144) (509) (513,550) (198)	29	(144)			1.00	h	(246)	0 0.5 0.3	sc c,i c,i	(513) (246) (550)
$1\alpha,25$-$(OH)_2$-23-yne-D_3	L	74 40	c r	(144) (186)	74	(143)	25 23	(143) (143)	3.00	h	(186)	0.9,0.1	c,i	(186)
$1\alpha,25$-$(OH)_2$-24R-F-D_3	M	87 140 115	c c r	(144) (208) (186)	151	(143)			0.30	h	(186)	>1 0.6,0.4	sc c,i	(209) (186)

APPENDIX I (Continued)

1α,25-(OH)$_2$-VITAMIN D$_3$ ANALOG NAME	ANA-LOG	VIT D RECEPTOR (non Human)			VIT D RECEPTOR (HL-60)		D-BINDING PROTEIN		CELL DIFFERENTIATION			CALCEMIC INDEX		
	CODE NAME	RCI	SPC	(ref)	RCI	(ref)	RCI	(ref)	ED50	(s)	(ref)	CI	(s)	(ref)
1α,25-(OH)$_2$-D$_3$-24,24,26,27-d$_8$	N	120	c	(144)			106	(143)	1.00	h	(186)	1.1.5	c,i	(186)
		100	r	(186)			107	(143)						
1α,25S,26-(OH)$_3$-D$_3$	O	88	c	(144)	92	(143)	4	(143)						
1α,23S,25-(OH)$_3$-D$_3$	P	12	c	(144)			29	(143)	0.14	h	(186)	0	c	(186)
1α,23R,25-(OH)$_3$-D$_3$	Q	11	c	(144)					0.50	h	(186)	0.0.1	c,i	(186)
		1	r	(186)										
1α,24R-(OH)$_2$-25F-D$_3$	R	95	c	(144)	85	(143)						1.0.02	c,i	(559)
		0.2	r	(186)										
1α,25-(OH)$_2$-26,27-F$_6$-23-yne-D$_3$	S	9	c	(144)										
1α,25R-(OH)$_2$-26-F$_3$-D$_3$	T	70	c	(144)					3.00	h	(186)	2.1 6	c,i	(186)
		190	r	(186)										
1α,25,28-(OH)$_3$-D$_3$	U	9	c	(144)	63	(143)	22	(143)				<0.06	c	(552)
1α,25-(OH)$_2$-16-Ene-23-yne-D$_3$	V	68	c	(144)	76	(143)	9	(143)	10.00	h	(186)	0.03,0.02	c,i	(349,188)
		70	c	(188)	75	(189)	30	(189)	2.00	h	(349)	0.03,0.02	i,c	(189)
		90	c	(127)	79	(188)	5	(143)	5.00	h	(189)	0 5.0	c,i	(187)
		50	r	(186)	60	(349)			10.00	h	(187)	0.05.0	c,i	(186)
		90	c	(349)										
		80	c	(189)										
		50	r	(187)										
1α,24R,25-(OH)$_2$-D$_3$	W	39	c	(144)			21	(143)	0.20	h	(186)	0.9,0 2	c,i	(186)
		2	r	(186)					0.25	h	(193)	1	i	(532)
		39	c	(190)										
1α,25-(OH)$_2$-26,27-F$_6$-23-ene-D$_3$	X	15	c	(144)										
25-(OH)-23-Yne-D$_3$	Y	0.03	c	(144)			2590	(143)	0.01	h	(186)	0 7.0	c,i	(186)
		<0.1	c	(127)			2970	(143)						
		0.06	r	(186)										
25-(OH)-26,27-F$_6$-23-yne-D$_3$	Z	0.18	c	(144)			470	(143)						
1α,25R-(OH)$_2$-22-Ene-26-F$_3$-D$_3$	AB	57	c	(144)										
1α,25S-(OH)$_2$-22-Ene-26-F$_3$-D$_3$	AC	40	c	(144)										

APPENDIX I (Continued)

1α,25-(OH)$_2$-VITAMIN D$_3$ ANALOG NAME	ANA-LOG	VIT D RECEPTOR (non Human)			VIT D RECEPTOR (HL-60)		D-BINDING PROTEIN		CELL DIFFERENTIATION			CALCEMIC INDEX		
	CODE NAME	RCI	SPC	(ref)	RCI	(ref)	RCI	(ref)	ED50	(s)	(ref)	CI	(s)	(ref)
1α,25R-(OH)$_2$-D$_3$-26,26,26-d$_3$	AD	90 83	c r	(144) (227)								1.4	c,i	(227)
1α,25S-(OH)$_2$-D$_3$-26,26,26-d$_3$	AE	129 150	c r	(144) (227)								0.9,1.5	c,i	(227)
1α,25R-(OH)$_2$-22-Ene-D$_3$-26,26,26-d$_3$	AF	133	c	(144)										
1α,25S-(OH)$_2$-22-Ene-D$_3$-26,26,26-d$_3$	AG	125	c	(144)										
1α,25-(OH)$_2$-D$_3$-26,26,26,27,27,27-d$_6$	AH													
1α,25-(OH)$_2$-24-Epi-D$_3$-26,26,26,27,27,27-d$_6$	AI													
1α,25-(OH)$_2$-D$_3$-23,23,24,24,26,26,26,27,27,27-d$_{10}$	AJ													
1α,25-(OH)$_2$-22-Ene-D$_3$-26,26,26,27,27,27-d$_6$	AK													
9(11)-Dehydro-3-deoxy-1,25-(OH)$_2$-D$_3$	AM	5	c	(144,61)										
2-Nor-1,3-seco-1,25-(OH)$_2$-D$_3$	AN	3	c	(144)										
2,4-Dinor-1,3-seco-1,25-(OH)$_2$-D$_3$	AP	2	c	(144)										
1,1-Dimethyl-2,4-dinor-1,3-seco-1,25-(OH)$_2$-D$_3$	AQ	0.11	c	(144)										
3-Deoxy-2-oxa-25-(OH)-D$_3$	AR	11	c	(144)										
24R,25-(OH)$_2$-D$_3$	AS	0.03 0.1	c c	(144) (196)			33800	(143)	<0.01 0.02 >0.01	u h h	(196) (481) (193)	<0.01 0 0.1	uc sc i	(196) (482,483) (532)
25-(OH)-16-Ene-23-yne-D$_3$	AT	0.4 <0.4 0.07 0.4 0.07	c c r c r	(144) (127) (186) (189) (187)	16	(143)	130 101	(143) (143)	0.01 0.05	h h	(186) (187)	0.7,0 0.02, 0.001 0.7,0	c,i i,c c,i	(186) (189) (187)
1-F-25-(OH)-16-ene-23-yne-D$_3$	AU	24	c	(189)	36	(143, 189)			4.00	h	(189)	0.0003, 0.0008	c,i	(189)
1α,25-(OH)$_2$-16-Ene-23-yne-D$_3$-26,26,26,27,27,27-d$_6$	AV	110 90	c c	(144) (189)	46 46	(143) (189)			3.00	h	(189)	0.01, 0.004	i,c	(189)
1-F-25-(OH)-16-ene-23-yne-D$_3$-26,26,26,27,27,27-d$_6$	AW	26	c	(189)	55	(143,189)			1.00	h	(189)	0.0002, 0.002	c,i	(189)

APPENDIX I (Continued)

1α,25-(OH)$_2$-VITAMIN D$_3$ ANALOG NAME	ANA-LOG	VIT D RECEPTOR (non Human)			VIT D RECEPTOR (HL-60)		D-BINDING PROTEIN		CELL DIFFERENTIATION			CALCEMIC INDEX		
	CODE NAME	RCI	SPC	(ref)	RCI	(ref)	RCI	(ref)	ED50	(s)	(ref)	CI	(s)	(ref)
A-Homo-3-deoxy-3,3-dimethyl-2,4-dioxa-25-(OH)-D$_3$	AX	4	c	(144)										
24-Nor-1α,25-(OH)$_2$-D$_3$	BA	67	c	(144)			20	(143)	0.07	h	(247)	0.02	c,i	(188)
		1	c	(188)					0.076	h	(193)			
25-Oxo-25-phospha-D$_3$	BB	11	c	(144)										
9(11)Dehydro-11-(4-hydroxymethylphenyl)-1,25(OH)$_2$-D$_3$	BD	3	c	(144)	0.75	(143)								
(23S,25S)-1α,25-(OH)$_2$-D$_3$-26,23-lactone	BF	12	c	(144)								0.3	sc	(536)
		8	c	(535-536)								0.25	c	(536)
1α,11β,25-(OH)$_3$-D$_3$	BG	5	c	(144)			7	(143)						
9(11)Dehydro-11(3'-hydroxypropyn-1'-yl)-1,25-(OH)$_2$-D$_3$	BH	3	c	(144)										
9(11)Dehydro-11(3'-acetoxypropyn-1'-yl)-1,25-(OH)$_2$-D$_3$	BI	0.7	c	(144)	1	(143)								
9(11)Dehydro-11(4-acetoxymethylphenyl)-1,25-(OH)$_2$-D$_3$	BJ	1	c	(144)										
Vitamin-D$_3$	BN	0.0001	c	(144)			2298	(143)						
25-(OH)-D$_3$	BO	0.15	c	(144)	2	(143)	66700	(143)						
1α-(OH)-D$_3$	BP	0.17	c	(144)	0.005	(143)	62	(143)						
(23R,25S)-1α,25-(OH)$_2$-D$_3$-26,23-lactone	BQ	0.3	c	(143)								0.02	c	(536)
		3	c	(501)								0.0	sc	(536)
		0.2	c	(535,536)										
(23R,25R)-1α,25-(OH)$_2$-D$_3$-26,23-lactone	BR	4	c	(144)								0.1	c	(536)
		2	c	(535-536)								0.0	sc	(536)
(23S,25R)-1α,25-(OH)$_2$-D$_3$-26,23-lactone (Natural Form)	BS	0.5	c	(144)	0.5	(211)	280	(501)	0.004	h	(211)	<0.01	sc	(195)
		1	c	(501)								0.03	c	(533)
		0.1	c	(533)								0.013	c	(536)
		0.2	c	(535-536)								0	sc	(536)
1α,24S-(OH)$_2$-22-Ene-26,27-dehydro-D$_3$	BT	111	c	(144)	131	(143)	49	(143)	1.00		(543)	<0.01		(543)
		240	u	(530)			70	(461)	1.00	u	(530)	<0.01	sc	(530)
		76	c	(522)			55	(143)	1.00	h	(127)	<0.01	sc	(461)
		100	c	(127)					1.00	u	(196)	<0.005	u	(196)
		100	c	(530)					1.00	u	(530)	0.05	sc	(530)
		100	c	(196)					1.00	h	(530)			
									1.00	h	(162)			
9(11)-Dehydro-1α,25-(OH)$_2$-D$_3$	BU	39	c	(144)			116	(143)						
		50	c	(61)			66	(143)						
1α,11α,25-(OH)$_3$-D$_3$	BW	9	c	(144)										
11β-Methoxy-1α,25-(OH)$_2$-D$_3$	BX	40	c	(144)	138	(143)	20	(143)						
							24	(143)						

APPENDIX I (Continued)

1α,25-(OH)$_2$-VITAMIN D$_3$ ANALOG NAME	ANA-LOG	VIT D RECEPTOR (non Human)			VIT D RECEPTOR (HL-60)		D-BINDING PROTEIN		CELL DIFFERENTIATION			CALCEMIC INDEX		
	CODE NAME	RCI	SPC	(ref)	RCI	(ref)	RCI	(ref)	ED50	(s)	(ref)	CI	(s)	(ref)
11α-Methoxy-1α,25-(OH)$_2$-D$_3$	BY	5	c	(144)	96	(143)								
25-(OH)-23-Oxa-D$_3$	BZ	6	c	(144)										
1α,24S,25-(OH)$_2$-D$_3$	CA	4	c	(190)										
3-Deoxy-1α,25-(OH)$_2$-D$_3$	CF	6	c	(144)								0.2,<0.1	c,i	(538)
		3	c	(61)								1	c	(531)
		6	c	(538)										
		13	c	(531)										
1α,24R-(OH)$_2$-D$_3$	CT	94	c	(144)	66	(143)			0.01	u	(196)	1	u	(196)
		100	c	(190,196)					1.00	h	(193)	1	c,i	(197)
1α,24S-(OH)$_2$-D$_3$	CU	9	c	(144)					1.00	h	(503)	1	c,i	(197)
		10	c	(190)					0.50	h	(193)			
1α,25-(OH)$_2$-24-Oxo-D$_3$	CW	98	c	(144)										
1α,23,25-(OH)$_3$-24-Oxo-D$_3$	CX	28	c	(144)										
1α-(OH)-25-Oxo-25-phospha-D$_3$	DA	0.4	c	(144)								0.003,	c,i	(188)
		0.3	c	(188)								0.001		
25-Oxo-26,27-dimethyl-25-phospha-26,27-dioxa-D$_3$	DB	0.02	c	(144)										
1α-(OH)-25-oxo-26,27-dimethyl-25-phospha-26,27-dioxa- D$_3$	DC	7	c	(144)			3	(143)	0.00	h	(537)	0.002,	c,i	(188)
		7	c	(188,537)								0.001		
												0.02,	c,i	(537)
												0.001		
22-(Meta-hydroxyphenyl)-1α,25-(OH)$_2$-D$_3$	DE	29	c	(144)	26	(143)	980	(143)						
22-(Para-hydroxyphenyl)-1α,25(OH)$_2$-D$_3$	DF	5	c	(144)	8	(143)	1980	(143)						
1α,25-(OH)$_2$-5,6-trans-D$_3$	DI	13	c	(179)								0.06,	c,i	(179)
												0.015		
25R,26-(OH)$_2$-D$_3$	DJ											<0.1	sc	(561)
25S,26-(OH)$_2$-D$_3$	DK						26792	(143)						
1α,25S,26-(OH)$_3$-D$_3$	DM						42	(143)						
1α,25R,26-(OH)$_3$-D$_3$	DN	80	c	(127)								0.25	sc	(195)
(23R,25S)-25-OH-D$_3$-26,23-lactone	EC	0.04	c	(501)			14100	(501)						

APPENDIX I (Continued)

1α,25-(OH)$_2$-VITAMIN D$_3$ ANALOG NAME	ANA-LOG	VIT D RECEPTOR (non Human)			VIT D RECEPTOR (HL-60)		D-BINDING PROTEIN		CELL DIFFERENTIATION			CALCEMIC INDEX		
	CODE NAME	RCI	SPC	(ref)	RCI	(ref)	RCI	(ref)	ED50	(s)	(ref)	CI	(s)	(ref)
(23S,25R)-25-OH-D$_3$-26,23-lactone	ED	0.0004	c	(501)			14100	(501)						
6-Fluoro-D$_3$	EM	0.001	c	(144)								0	c	(500,554)
		0.3	c	(500)										
1α,25-(OH)$_2$-16-Ene-23-yne-26,27-F$_6$-D$_3$	EO	46	c	(144)	31	(143,188)	15	(143)	80.00	h	(188,189)	0.06	c,i	(188)
		45	c	(188)			25	(143)						
25-(OH)-16-Ene-23-yne-26,27-F$_6$-D$_3$	EP	0.6	c	(143)	2.2	(143)	10	(143)				0.001,	c,i	(188)
		0.6	c	(188)								0.02		
1α-F-25-(OH)-16-ene-23-yne-26,27-F$_6$-D$_3$	EQ	20	c	(144)	9	(143,188)	72	(143)	1.00	h	(189)	0.005,	c,i	(188)
		5	c	(188)			105	(143)	5.70	h	(188)	0.002		
1α,25-(OH)$_2$-24a-Homo-D$_3$	ER	53	c	(144)	54	(143)	8	(143)	10.00	h	(249,250)	0.2,1	i,c	(249)
		100	r	(249)	100	(249)			10.00	h	(247)	<0.1	i,c	(250)
		100	c	(247)					7.6	h	(193)			
1α,25-(OH)$_2$-24a-Dihomo-D$_3$	ES	25	c	(144)	16		8	(143)	10.00	h	(250)	<0.01	c,i	(250)
		3	r	(250)	3	(250)								
22(m-methylphenyl)-23,24,25,26,27-pentanor-1α-(OH)-D$_3$	ET	1	c	(144)	4									
22-Oxa-1α,25-(OH)$_2$-D$_3$	EU	15	c	(144)	53		23	(143)	10.00	h	(510)	0.01	sc	(231)
		12	c	(233)			0.2	(233)	0.00					
		100	r	(235)	10	(231-233)	<0.1	(231)	10.00	h	(236)	0.004	u	(238)
		10		(232)			<0.2	(232)	10.00	h	(556)	<0.001	sc	(156)
		7	c	(510)			22	(143)				<0.01	i,sc	(237)
		100	m	(234)			<0.20	(291)						
22(m-(dimethylhydroxymethyl)phenyl)-23,24,25,26,27-pentanor-1α-(OH)-D$_3$	EV	62	c	(144)	59	(143)	13.4	(143)						
							25	(143)						
1α,25-(OH)$_2$-22-Ene-D$_3$	EX	86	c	(144)			40	(143)						
25-(OH)-23-Ene-D$_3$	EY						24498	(143)	0.01	h	(193)			
1α,25-(OH)$_2$-16,23(E)-diene-D$_3$	EZ	80	r	(186,187)			17	(143)	3.00	h	(186,187)	1.4,0.8	c,i	(187)
14-Epi-1α,25-(OH)$_2$-D$_3$	GE	15	c	(144)			12	(143)						
14-Epi-1α,25-(OH)$_2$-pre-D$_3$	GF	2	c	(144)			2	(143)						
3-Deoxy-3-thia-1α,25-(OH)$_2$-D$_3$	HA	15	c	(538)								0.2,<0.1	c,i	(538)
3-Deoxy-3-thia-1β,25-(OH)$_2$-D$_3$	HB	1	c	(538)								<0.2,<0.1	c,i	(538)
1α,25-(OH)$_2$-pre-D$_3$-9,14,19,19,19-d$_5$	HF	10	c	(92)			9	(143)	<0.05	h	(92)	<0.05	oc	(92)
							7	(92)						
1α,25-(OH)$_2$-D$_3$-9,9,14,19,19-d$_5$	HG	90	c	(144)			51	(143)	0.90	h	(92)	>1	oc	(92)

APPENDIX I (Continued)

1α,25-$(OH)_2$-VITAMIN D_3 ANALOG NAME	ANA-LOG	VIT D RECEPTOR (non Human)			VIT D RECEPTOR (HL-60)		D-BINDING PROTEIN		CELL DIFFERENTIATION			CALCEMIC INDEX		
	CODE NAME	RCI	SPC	(ref)	RCI	(ref)	RCI	(ref)	ED50	(s)	(ref)	CI	(s)	(ref)
		92	c	(92)			60 95	(143) (92)						
1β,25-$(OH)_2$-3-epi-D_3	HH	0.22	c	(144)			6570	(143)						
1α,25-$(OH)_2$-6,7-Dehydro-pre-D_3	HI	3	c	(144)										
1α,25-$(OH)_2$-3-epi-D_3	HJ	24	c	(144)			800	(143)						
1β,25-$(OH)_2$-6,7-Dehydro-3-epi-pre-D_3	HK	0.05	c	(144)										
1β,25-$(OH)_2$-D_3	HL	0.00 <0.8 <0.1	c c	(144) (132) (506)			449	(143)				<0.001	i,c	(496)
1α,25-$(OH)_2$-16-Ene-D_3	HM	165 240 250	c r r	(144) (186,187) (171)	150	(143)	2	(143)	5.00	h	(186,187)	0,0.6 0	c,i	(187) (171)
25-(OH)-16-Ene-D_3	HN	0.7 0.01 0.2 0.2	c c r r	(144) (127) (186) (187)	0.06 0.03	(143) (143)	489 233	(143) (143)	0.03 0.01	h h	(186) (187)	1,0	c,i	(187)
25-(OH)-16,23-Diene-D_3	HO	0.1 0.1 0.1	r r r	(186) (187) (171)			524	(143)	0.005 0.02	h h	(186) (187)	2.5,0 2.5	c,i c	(187) (171)
1α,2,25-$(OH)_3$-D_3	HP	70	c	(144)										
(22S)-1α,25-$(OH)_2$-22,23-Diene-D_3	HQ	21	c	(144)			25	(143)						
(22R)-1α,25-$(OH)_2$-22,23-Diene-D_3	HR	52	c	(144)			48 47	(143) (143)						
1α,18,25-$(OH)_3$-D_3	HS	25 20	c b	(144) (184)					0.50	h	(184)	<1	c	(184)
1α,18-$(OH)_2$-D_3	HV	0.02	c	(144)										
18-Acetoxy-1α,25-$(OH)_2$-D_3	HW	0.04	c	(144,183)								0.001, 0.01	c,i	(183)
18-Acetoxy-1α-(OH)-D_3	HZ	0.02	c	(144,183)								<0.0005 <0.003	c c	(183) (183)
23-(m-(Dimethylhydroxymethyl)phenyl)-22-yne-24,25,26,27-tetranor-1α-(OH)-D_3	IB	1	c	(144)										

APPENDIX I (Continued)

1α,25-(OH)$_2$-VITAMIN D$_3$ ANALOG NAME	ANA-LOG	VIT D RECEPTOR (non Human)			VIT D RECEPTOR (HL-60)		D-BINDING PROTEIN		CELL DIFFERENTIATION			CALCEMIC INDEX		
	CODE NAME	RCI	SPC	(ref)	RCI	(ref)	RCI	(ref)	ED50	(s)	(ref)	CI	(s)	(ref)
24a,26a,27a,-Trihomo-22,24-diene-1αa,25-(OH)$_2$-D$_3$	IC	17	c	(144)										
20-Epi-22-oxa-24a,26a,27a-trihomo-1α,25-(OH)$_2$-D$_3$	ID	25	c	(144)					20000	u	(244)	1.3	r	(244,160)
		120	c	(244)										
20-Epi-1α,25-(OH)$_2$-D$_3$	IE	147	c	(144)					27.00	u	(244)	2.3	u	(244,160)
		120	c	(244)										
20-Epi-24a,26a,27a-trihomo-1α,25-(OH)$_2$-D$_3$	IF	23	c	(144)					200.0	u	(244)	1.2	r	(244,160)
		100	c	(244)										
18-oxo-1α,25-(OH)$_2$-D$_3$	IG	26	c	(144)										
3-Deoxy-3-thia-1α,25-(OH)$_2$D$_3$-3β-oxide	II	27	c	(144)										
5,6-trans-3-Deoxy-3-thia-1α,25-(OH)$_2$-D$_3$-3β-oxide	IK	4	c	(144)										
24a-Homo-22,24(24a)-diene-1α,25(OH)$_2$D$_3$	IP	26	c	(144)										
24a-Dihomo-1α,22R,25-(OH)$_3$-D$_3$	IQ	2	c	(144)										
8,(14a)-homo-1α,25-(OH)$_2$D$_3$	KB						20	(182)	1.00	h	(182)	0.1	sc	(182)
23-oxa-1α,25-(OH)$_2$-D$_3$	KD								0.20	h	(242)			
1α-(hydroxymethyl)-25-OH-D$_3$	ZAA	<0.1	c	(173)					<.001		(563)	<0.001	c	(173)
1β-(hydroxymethyl)-3α,25-(OH)$_2$-D$_3$	ZAB	<0.1	c	(173)								<0.001	c	(173)
2β-(3-hydroxypropoxy)-1α,25-(OH)$_2$-D$_3$	ZAC	10	c	(232)			200	(232)				1	i,c	(516)
		12	c	(516)			200	(516)						
1α,25-(OH)$_2$-24(S)-5,6-t-D$_3$	ZAD	50	c	(198)										
1α,25-(OH)$_2$-24(R)-5,6-t-D$_3$	ZAE	1	c	(198)										
11α-phenyl-1α,25-(OH)$_2$-D$_3$	ZAF	<1	r	(140)			200	(140)	0.03	h	(140)	<0.01	c	(140)
11β-phenyl-1α,25-(OH)$_2$-D$_3$	ZAG	5	r	(140)			150	(140)	0.07	h	(140)	<0.01	c	(140)
11α-dimethylaminophenyl-1α,25-(OH)$_2$-D$_3$	ZAH	1	r	(140)			1	(140)	<.001	h	(140)	<0.001	c	(140)
11α-methyl-1α,25-(OH)$_2$-D$_3$	ZAI	230	r	(140)			340	(140)	1.13	h	(140)	0.4	c	(140)
11β-methyl-1α,25-(OH)$_2$-D$_3$	ZAJ	37	r	(140)			86	(140)	0.19	h	(140)	0.02	c	(140)
11α-hydroxymethyl-1α,25-(OH)$_2$-D$_3$	ZAK	<1	r	(140)			107	(140)	0.07	h	(140)	<0.01	c	(140)
11α-fluoromethyl-1α,25-(OH)$_2$-D$_3$	ZAL	75	r	(140)			250	(140)	0.63	h	(140)	0.1	c	(140)
11α-chloromethyl-1α,25-(OH)$_2$-D$_3$	ZAM	12	r	(140)			160	(140)	0.28	h	(140)	0.1	c	(140)
11α-ethyl-1α,25-(OH)$_2$-D$_3$	ZAN	9	r	(140)			272	(140)	0.43	h	(140)	0.02	c	(140)
11α-(2-hydroxyethyl)-1α,25-(OH)$_2$-D$_3$	ZAO	0.1	r	(140)			115	(140)	0.07	h	(140)	<0.01	c	(140)
11β-(2-hydroxyethyl)-1α,25-(OH)$_2$-D$_3$	ZAP	<1	r	(140)			40	(140)	0.05	h	(140)	<0.01	c	(140)
11α-vinyl-1α,25-(OH)$_2$-D$_3$	ZAQ	24	r	(140)			380	(140)	0.81	h	(140)	<0.01	c	(140)

APPENDIX I (Continued)

— 1α,25-$(OH)_2$-VITAMIN D_3 ANALOG NAME	ANA-LOG	VIT D RECEPTOR (non Human)			VIT D RECEPTOR (HL-60)		D-BINDING PROTEIN		CELL DIFFERENTIATION			CALCEMIC INDEX		
	CODE NAME	RCI	SPC	(ref)	RCI	(ref)	RCI	(ref)	ED50	(s)	(ref)	CI	(s)	(ref)
11α-ethynyl-1α,25-$(OH)_2$-D_3	ZAR	210	r	(140)			25	(140)	2.26	h	(140)	0.16	c	(140)
11α-[11'(R)-oxacyclopropyl]-1α,25-$(OH)_2$-D_3	ZAS	2	r	(140)			80	(140)	<0.01	h	(140)			
11α-[11'(S)-oxacyclopropyl]-1α,25-$(OH)_2$-D_3	ZAT	1	r	(140)			62	(140)	<0.01	h	(140)			
1α,25-$(OH)_2$-13-vinyl-18-nor-D_3	ZAU	25	b	(184)			100	(184)	0.50	h	(184)	<1	c	(184)
25-(OH)-16,23(Z)-diene-D_3	ZAW	0.8	r	(187)					<0.01	h	(187)			
1α,25-$(OH)_2$-16,23(Z)-diene-D_3	ZAX	145	r	(187)			17	(187)	1.00	h	(187)			
1α,25-$(OH)_2$-18-methyl-D_3	ZAY	100	b	(184)			100	(184)	0.50	h	(184)	<1	c	(184)
1α,25-$(OH)_2$-19-nor-pre-D_3	ZAZ	1	p	(176)			6	(176)	0.01	h	(176)	0	sc	(176)
1α,25-$(OH)_2$-19-nor-D_3	ZBA	30	p	(176)			20	(176)	1.00	h	(176)	<0.1	sc	(176)
25,26-epoxy-23-yne-19-nor-1α-(OH)-D_3	ZBB	20	p	(204)			1	(204)	0.12	h	(204)	<0.01	c	(204)
20-epi-24-homo-1α,25-$(OH)_2$-D_3	ZBC	110	c	(244)					200.0	u	(244)	5	r	(244,160)
20-epi-22-oxa-1α,25-$(OH)_2$-D_3	ZBD	2	c	(244)					4.00	c	(244)	0.1	r	(244)
20-epi-22-oxa-24-homo-1α,25-$(OH)_2$-D_3	ZBE	8	c	(244)					1176	u	(244)	3	r	(244)
20-epi-22-oxa-24-dihomo-1α,25-$(OH)_2$-D_3	ZBF	35	c	(244)					400.0	u	(244)	1.4	r	(244)
20-epi-22-oxa-24-dihomo-26,27-dihomo-1α,25-$(OH)_2$-D_3	ZBG	60	c	(244)					400.0	u	(244)	0.8	r	(244)
20-epi-23-oxa-24a,24b-dihomo-1α,25-$(OH)_2$-D_3	ZBH	60	c	(548)					1000	u	(548)	0.6	u	(548)
25,26-epoxy-23-yne-20-epi-1α-(OH)-D_3	ZBI	302	p	(204)			0	(204)	20.00	h	(204)	0.03	c	(204)
1α-(OH)-20-oxa-21-nor-D_3	ZBJ	0.1	c	(240)					1.00 0.20 <0.05	h h h	(240) (510) (236)			
1α,25-$(OH)_2$-20-oxa-21-nor-D_3	ZBK	0.1	c	(240)					1.00 0.20 0.1	h h h	(240) (556,510) (236)	0	i,c	(240)
22-oxa-1α-(OH)-D_3	ZBL	1	c	(510)					<0.10	h	(236)			
1α,24(S)-$(OH)_2$-22-oxa-D_3	ZBM								10.00	h	(239)			
1α,24(S)-$(OH)_2$-22-oxa-26,27-dimethyl-D_3	ZBN								10.00	h	(239)			
1α,25-$(OH)_2$-22-oxa-26,27-dimethyl-D_3	ZBO								30.00	h	(239)			
22-(OH)-D_3	ZBP	<0.1	c	(505)			500	(505)				<0.001	cbp	(505)
1α-(OH)-22-oxo-D_3	ZBQ								0.01	h	(193)			
23,24,25,26,27-pentanor-1,22-$(OH)_2$-D_3	ZBR								0.005	h	(193)			

APPENDIX I (Continued)

1α,25-(OH)$_2$-VITAMIN D$_3$ ANALOG NAME	ANA-LOG	VIT D RECEPTOR (non Human)			VIT D RECEPTOR (HL-60)		D-BINDING PROTEIN		CELL DIFFERENTIATION			CALCEMIC INDEX		
	CODE NAME	RCI	SPC	(ref)	RCI	(ref)	RCI	(ref)	ED50	(s)	(ref)	CI	(s)	(ref)
1α-(OH)-22-E-ene-D$_3$	ZBS	0.1	c	(504)					0.02	h	(193)			
1α-(OH)-22-Z-ene-D$_3$	ZBT								<0.01	h	(193)			
1α,25(OH)$_2$-22-ene-24-homo-D$_3$	ZBU	100	r	(249)					10.00	h	(249,247)	1,<0.2	c,i	(249)
		100	c	(247)										
1α,25-(OH)$_2$-22-ene-24,24-dihomo-D$_3$	ZBV	4		(521)	3	(250)			100.0	h	(521)	0	sc	(521)
		3	r	(250)					10.00	h	(250)	<0.001	c	(250)
22-dehydro-24,24,24-trihomo-1α,25-(OH)$_2$-D$_3$	ZBW	<1		(521)	0.8	(250)			1.00	h	(521)	0	sc	(521)
		0.8	r	(250)					0.5	h	(250)	0	c	(250)
26-homo-22-dehydro-1α,25(R)-(OH)$_2$-D$_3$	ZBX	100	c	(509,247)	100	(249)			10.00	h	(249,247)	1	sc	(509)
		100	r	(249)					1.40	h	(201)	1,>1	c	(249)
1α-(OH)-22-ene-24-oxo-26,27-dehydro-D$_3$	ZBY	0.3	c	(522)					0.01	u	(522)			
(24S,25S)-25,26-epoxy-22-ene-1α,24-(OH)$_2$-D$_3$	ZBZ	8	p	(204)			3	(204)	0.15	h	(204)	0.01	c	(204)
(24S,25R)-25,26-epoxy-22-ene-1α,24-(OH)$_2$-D$_3$	ZCA	8	p	(204)			2	(204)	0.30	h	(204)	0.01	c	(204)
(22E,24R)-1α,24-(OH)$_2$-22-dehydro-D$_3$	ZCB	10	c	(203)								0.89	sc	(203)
(22E,24S)-1α,24-(OH)$_2$-22-dehydro-D$_3$	ZCC	100	c	(203)								0.95	sc	(203)
24,25-epoxy-22-yne-1α-(OH)-D$_3$	ZCD	2	p	(204)			5	(204)	0.03	h	(204)	<0.01	c	(204)
23,24-dinor-1,25-(OH)$_2$-D$_3$	ZCE								0.004	h	(193)			
23-oxa-24a,24b-dihomo-1α,25-(OH)$_2$-D$_3$	ZCF	<100	c	(548)					<1.00	u	(548)			
23-thia-1α,25-(OH)$_2$-D$_3$	ZCG								0.20	h	(242)			
23-aza-1α,25-(OH)$_2$-D$_3$	ZCH								0.13	h	(242)			
24,25-epoxy-26,27-dinor-23,23-dimethyl-1α-(OH)-D$_3$	ZCI	1	p	(204)			0	(204)	0.00	h	(204)	<0.001	c	(204)
1α,23-(OH)$_2$-25,26-dehydro-D$_3$	ZCJ													
23-keto-25-(OH)-D$_3$	ZCK	2	b	(508)			13600	(508)				20.0	c	(508)
23(S)-OH-26,27-F$_6$-1α,25-(OH)$_2$-D$_3$	ZCL	30	c	(230)					10.00	h	(229)	1	c	(230)
24,25,26,27-tetranor-1,23-(OH)$_2$-D$_3$	ZCM								0.004	h	(193)			
23(S),25(R)-1α,25-(OH)$_2$-D$_3$-26,23-lactol	ZCN											<0.1	sc	(195)
1α,25-(OH)$_2$-16,23(Z)-diene-D$_3$	ZCP	145	r	(187)					1.00	h	(187)			
25,26-epoxy-23-yne-1α-(OH)-D$_3$	ZCQ	30	p	(204)			6	(204)	2.40	h	(204)	0.01	c	(204)
1α,25-(OH)$_2$-24,26,27-trihomo-D$_3$	ZCS	18	c	(244)					5.00	u	(244)	0.2	u	(244)
22-oxa-24,26,27-trihomo-1α,25-(OH)$_2$-D$_3$	ZCT	30	c	(244)					20.00	u	(244)	30	r	(244)
24,24-difluoro-24-homo-1α25-(OH)$_2$-D$_3$	ZCU	28	c	(218)			0.15	(218)	10.00	h	(217)	1,<1	c,i	(218)
24R-(OH)-25-F-D$_3$	ZCV	0.2	c	(215,560)								>0.002	r	(215)
26,27-F6-1α,24-(OH)$_2$-D$_3$	ZCW	60	c	(223)	60	(223)			10.00	h	(223)			

APPENDIX I (Continued)

1α,25-$(OH)_2$-VITAMIN D_3 ANALOG NAME	ANA-LOG	VIT D RECEPTOR (non Human)			VIT D RECEPTOR (HL-60)		D-BINDING PROTEIN		CELL DIFFERENTIATION			CALCEMIC INDEX		
	CODE NAME	RCI	SPC	(ref)	RCI	(ref)	RCI	(ref)	ED50	(s)	(ref)	CI	(s)	(ref)
(24S,25S)-25,26-epoxy-1α,24-$(OH)_2$-D_3	ZCX	5	p	(204)			3	(204)	0.07	h	(204)	0.02	c	(204)
(24R,25R)-25,26-epoxy-1α,24-$(OH)_2$-D_3	ZCY	20	p	(204)			73	(204)	0.12	h	(204)	0.01	c	(204)
(24S,25R)-25,26-epoxy-1α,24-$(OH)_2$-D_3	ZCZ	10	p	(204)			7	(204)	0.44	h	(204)	0.03	c	(204)
(24R,25S)-25,26-epoxy-1α,24-$(OH)_2$-D_3	ZDA	9	p	(204)			30	(204)	0.33	h	(204)	0.01	c	(204)
(24R,25S)-25,26-epoxy-27-nor-1α,24-$(OH)_2$-D_3	ZDB	3	p	(204)			21	(204)	<0.01	h	(204)	<0.01	c	(204)
(24S,25R)-25,26-epoxy-27-nor-1α,24-$(OH)_2$-D_3	ZDC	2	p	(204)			5	(204)	<0.01	h	(204)	<0.01	c	(204)
24,25-epoxy-1α-(OH)-D_3	ZDD	3	p	(204)			27	(204)	0.06	h	(204)	0	c	(204)
24-ene-D_3	ZDE											0	sc	(549)
1α-(OH)-24-ene-D_3	ZDF	0.2	c	(190)										
24,24-difluoro-1α,25$(OH)_2$-26,27-dimethyl-D_3	ZDH	50	c	(218)			4	(218)				1,<1	c,i	(218)
22,23-dihydro-24-epi-1α,25-$(OH)_2$-D_3	ZDK								1.00	h	(248)	0.50	sc,c	(246)
25,26,27-trinor-1α,25-$(OH)_2$-D_3	ZDL								0.01	h	(193)			
25-aza-D_3	ZDM													
25,26-epoxy-1α-(OH)-D_3	ZDN	27	p	(204)			76	(204)	37.00	h	(204)	0.14	c	(204)
1α-(OH)-25-hydroxymethyl-D_3	ZDO								0.14	h	(193)			
1α-(OH)-25-F-D_3	ZDP	0.3	c	(507)								0,0	c	(186)
												0.02	c,i	(507)
												0	sc	(490)
1α,25-F2-D_3	ZDQ													
1α,25-$(OH)_2$-26-homo-D_3	ZDR	100	r	(249)	100	(249)			10.00	h	(249)	1,>1	c,i	(249)
1α,25-$(OH)_2$-26-homo-D_3		100	c	(247)					10.00	h	(247)			
									7.60	h	(193)			
26,27-dimethyl-1α,25-$(OH)_2$-D_3	ZDV	50	c	(515,562)	120	(252,253)	5	(515, 562)	2.50	h	(217)	>1	sc	(488)
		100	c	(254)					1.00	h	(252,253)	0.5	cbp	(515)
		110	c	(568)										
							10	(252)	2.50	h	(247)	>1,1	i,c	(254)
							<100	(254)				>1	c,i	(562)
												<1	sc	(247)
26,27-diethyl-1α,25-$(OH)_2$-D_3	ZDW	16	c	(254)	20	(252,253)	<1	(252)	10.00	h	(217)	<1	c,i	(254)
		25	c	(568)										
							<10	(254)	0.20	h	(252)	0		(247)
									0.5	h	(253)			
									10.00	h	(247)			

APPENDIX I (Continued)

1α,25-(OH)$_2$-VITAMIN D$_3$ ANALOG NAME	ANA-LOG	VIT D RECEPTOR (non Human)			VIT D RECEPTOR (HL-60)		D-BINDING PROTEIN		CELL DIFFERENTIATION			CALCEMIC INDEX		
	CODE NAME	RCI	SPC	(ref)	RCI	(ref)	RCI	(ref)	ED50	(s)	(ref)	CI	(s)	(ref)
26,27-dipropyl-1α,25-(OH)$_2$-D$_3$	ZDX	3 5	c c	(254) (568)	3	(252,253)	<1 <10	(252) (255)	0.00	h	(252,253)	<<1.0	c,i	(254)
1α,23(S),25(R),26-(OH)$_4$D$_3$	ZDY											0.05	sc	(195)
1α-(OH)-26,27-F$_6$-D$_3$	ZDZ	1	c	(223)	1	(223)			1.00	h	(223)			
25-(OH)-26,27-F$_6$-D$_3$	ZEA								0.08	h	(193)			
26,27-dinor-1α,25-(OH)$_2$D$_3$	ZEB								0.01	h	(247,193)			
1α-(OH)-24-oxo-26,27-dehydro-D$_3$	ZEC													
23-oxa-26,27-dimethyl-1α,25-(OH)$_2$D$_3$	ZED	50		(574)			<2	(574)	1	h	(574)	<0.2	u	(574)
20-ene-23-oxa-26,27-dimethyl-1α,25-(OH)$_2$D$_3$	ZEE	30	p	(574)			<2	(574)	0.3	h	(574)	0.2	u	(574)
20,21-methano-23-oxa-26,27-dimethyl-1α,25-(OH)$_2$D$_3$	ZEF	0	p	(574)			<2	(574)	0.2	h	(574)	1	u	(574)
20-methyl-23-oxa-26,27-dimethyl-1α,25-(OH)$_2$D$_3$	ZEG	50	p	(574)			<2	(574)	0.5	h	(574)	0.2	u	(574)
22-ene-26-methyl-1α,25S(OH)$_2$D$_3$	ZEH	150	c	(575)					2.1	h	(576)	2.12i/1.6	c	(577)
22-ene-26,27-dimethyl-1α,25-(OH)$_2$D$_3$	ZEI	80	h	(578)			0.75-r <1	(205) (578)	60	h	(578)			
22-ene-24-homo-26,27-dimethyl-1α,25-(OH)$_2$D$_3$	ZEJ								20	u	(485)	0.2	u	(485)
20-epi-22-ene-24-homo-26,27-dimethyl-1α,25-(OH)$_2$D$_3$	ZEK								1000	u	(485)	1.2	u	(485)
22-yne-26,27-dimethyl-1α,25-(OH)$_2$D$_3$	ZEL	90	c	(486)					3.5	u	(486)			
22-yne,24-homo-26,27-dimethyl-1α,25-(OH)$_2$D$_3$	ZEM								1.8	u	(486)			
22-yne-24-dihomo-26,27-dimethyl-1α,25-(OH)$_2$D$_3$	ZEN	2	c	(486)					0.1	u	(486)			
20-epi-22-yne-26,27-dimethyl-1α,25-(OH)$_2$D$_3$	ZEO	30	c	(486)					4	u	(486)			
20-epi-22-yne-24-homo-26,27-dimethyl-1α,25-(OH)$_2$D$_3$	ZEP	40	c	(486)					280	u	(486)			
20-epi-22-yne-24-dihomo-26,27-dimethyl-1α,25-(OH)$_2$D$_3$	ZEQ	7	c	(486)					490	u	(486)	0.6	u	(486)
20-epi-22-yne-24-trihomo-26,27-dimethyl-1α,25-(OH)$_2$D$_3$	ZER	80	c	(486)					25	u	(486)			
17(20)E-ene-22-yne-24-homo-26,27-dimethyl-1α,25-(OH)$_2$D$_3$	ZES	0.1	c	(486)					2.5	u	(486)			
17(20)Z-ene-22-yne-26,27-dimethyl-1α,25-(OH)$_2$D$_3$	ZET	7	c	(486)					32	u	(486)			
17(20)Z-ene-22-yne-24-homo-26,27-dimethyl-1α,25-(OH)$_2$D$_3$	ZEU	20	c	(486)					710	u	(486)	0.7	u	(486)
17(20)Z-ene-22-yne-24-dihomo-26,27-dimethyl-1α,25-(OH)$_2$D$_3$	ZEV	2	c	(486)					290	u	(486)	0.25	u	(486)

APPENDIX I (Continued)

1α,25-$(OH)_2$-VITAMIN D_3 ANALOG NAME	ANA-LOG	VIT D RECEPTOR (non Human)			VIT D RECEPTOR (HL-60)		D-BINDING PROTEIN		CELL DIFFERENTIATION			CALCEMIC INDEX		
	CODE NAME	RCI	SPC	(ref)	RCI	(ref)	RCI	(ref)	ED50	(s)	(ref)	CI	(s)	(ref)
20-ene-22-yne-26,27-dimethyl-1α,25-$(OH)_2D_3$	ZEW	90	c	(486)					1.4	u	(486)			
20-ene-22-yne-24-homo-26,27-dimethyl-1α,25-$(OH)_2D_3$	ZEX	3	c	(486)					1.2	u	(486)			
26,27-dimethyl-1α,20,25-$(OH)_3D_3$	ZEY	20	?	(542)					100	u	(542)	0.06	u	(542)
24-homo-26,27-dimethyl-1α,20,25-$(OH)_3D_3$	ZEZ	0.6	?	(542)					9	u	(542)	<0.005	u	(542)
20-methoxy-26,27-dimethyl-1α,25-$(OH)_2D_3$	ZFA	50	?	(542)					8000	u	(542)	0.3	u	(542)
20-methoxy-24-homo-26,27-dimethyl-1α,25-$(OH)_2D_3$	ZFB	1	?	(542)					300	u	(542)	0.06	u	(542)
20-ethoxy-26,27-dimethyl-1α,25-$(OH)_2D_3$	ZFC	7	?	(542)					5000	u	(542)	0.1	u	(542)
20-ethoxy-24-homo-26,27-dimethyl-1α,25-$(OH)_2D_3$	ZFD	1	?	(542)					300	u	(542)			
26,27-dimethyl-1α,22S,25-$(OH)_3D_3$	ZFE	6	c	(532)					100	u	(486)	7.1	u	(532)
24-homo-26,27-dimethyl-1α,22S,25-$(OH)_3D_3$	ZFF	2	c	(532)					20	u	(532)	0.8	u	(532)
24-dihomo-26,27-dimethyl-1α,22S,25$(OH)_3D_3$	ZFG	<0.5	c	(532)					100	u	(532)			
23-yne-24-dihomo-26,27-dimethyl-1α,22S,25-$(OH)_3D_3$	ZFH	0.5	c	(532)					100	u	(532)			
23-yne-24-trihomo-26,27-dimethyl-1α,22S,25-$(OH)_3D_3$	ZFI	<0.3	c	(532)					1	u	(532)			
23-yne-26,27-dimethyl-1α,22S,25-$(OH)_3D_3$	ZFJ	<3	c	(532)					1	u	(532)			
23-yne-24-homo-26,27-dimethyl-1α,22S,25-$(OH)_3D_3$	ZFK	0.5	c	(532)					0.1	u	(532)			
23-yne-24-dihomo-26,27-dimethyl-1α,22S,25-$(OH)_3D_3$	ZFL	<0.5	c	(532)					0.5	u	(532)			
23-yne-24-trihomo-26,27-dimethyl-1α,22S,25-$(OH)_3D_3$	ZFM	<0.3	c	(532)					<0.01	u	(532)			
22R-methoxy-23-yne-26,27-dimethyl-1α,25-$(OH)_2D_3$	ZFN	40	c	(532)					1000	u	(532)	12	u	(532)
22R-methoxy-23-yne-24-homo-26,27-dimethyl-1α,25-$(OH)_2D_3$	ZFO	10	c	(532)			110	(532)	200	u	(532)			
23-oxa-26,27-diethyl-1α,25-$(OH)_2D_3$	ZFP	10	p	(574)			<2	(574)	0.25	h	(574)	0.01	u	(574)
20-ene-23-oxa-26,27-diethyl-1α,25-$(OH)_2D_3$	ZFQ	25	p	(574)			<2	(574)	0.2	h	(574)	0.01	u	(574)
20,21-methano-23-oxa-26,27-diethyl-1α,25-$(OH)_2D_3$	ZFR	50	p	(574)			<2	(574)	0.1	h	(574)	0.003	u	(574)
20-epi-22-oxa-24-dihomo-26,27-diethyl-1α,25-$(OH)_2D_3$	ZFS	60	c	(525)					400	u	(525)	0.8	u	(525)
26,27-diethyl-1α,20,25-$(OH)_3D_3$	ZFT	0.5	?	(542)					<0.1	u	(542)			
20-methoxy-26,27-diethyl-1α,25-$(OH)_2D_3$	ZFU	0.9	?	(542)					0.1	u	(542)			
22-ene-26,27-dehydro-1α,24R-$(OH)_2D_3$	ZFV								0.01	u	(485)	<0.01	u	(485)
20-epi-22-ene-26,27-dehydro-1α,24S-$(OH)_2D_3$	ZFW								0.1	u	(485)	<0.01	u	(485)
20-epi-22-ene-26,27-dehydro-1α,24R-$(OH)_2D_3$	ZFX								1	u	(485)	<0.01	u	(485)
24-dihomo-26,27-diethyl-1α,25-$(OH)_2D_3$	ZFY								0.01	u	(489)			
20-epi-22-oxa-24-homo-26,27-diethyl-1α,25-$(OH)_2D_3$	ZFZ								100	u	(495)	<0.26	u/sc	(495)
22-oxa-26,27-diethyl-1α,25-$(OH)_2D_3$	ZGA								1	h	(439)			

The numerical values in the table for Relative Competitive Index (RCI) for the nuclear VDR and the RCI for the vitamin D binding protein (DBP) tabulate the data for the indicated parameter in relation to the result for $1\alpha,25(OH)_2D_3$ which is normalized to 100; thus the data values represent percentages of the $1\alpha,25(OH)_2D_3$ value. The key to the data entries is as follows. The RCI measures the relative ability of an analog under *in vitro* conditions to compete with $[^3H]1\alpha,25(OH)_2D_3$ for binding to the nuclear $1\alpha,25(OH)_2D_3$ receptor (VDR) (141) or the plasma vitamin D transport protein (DBP) (142). The cell differentiation data are related to that for $1\alpha,25(OH)_2D_3$ for which the value is set at 1.00. The calcemic index data are set at a value of 1.00 relative to that of $1\alpha,25(OH)_2D_3$.

SPC indicates the species of origin of the nVDR where c = chick intestine, r = rat intestine, b = bovine thymus, p = pig intestine, m = MCF-7 human breast cancer cells, z = rat osteosarcoma ROS 17/2.8 cells. For the DBP, all data are obtained from the human protein. The cell differentiation data are obtained in human transformed cell lines where h = HL-60 cells, u = U-937. The calcemic index is a measure of the relative ability of an analog to generate a "calcemic" response, which is defined differently depending upon the assay being conducted; c or i = a measure of intestinal Ca^{2+} absorption (ICA) or bone Ca^{2+} mobilizing activity (BCM) in the vitamin D-deficient chick (146,553); b = bone resorption; cbp = induction of the vitamin D-induced calcium binding protein, calbindin-D_{28k}; sc = elevation serum Ca^{2+} = concentrations; oc = an increase in serum osteocalcin levels; r = an increase in urinary Ca^{2+} excretion. Reference numbers are indicated in parentheses, and refer to regerence numbers in Boleillon et.al,1995.

APPENDIX II

NCI Common Toxicity Criteria Used to Grade Toxicities

Toxicity Grade	**0**	**1**	**2**	**3**	**4**
Blood/Bone Marrow					
WBC	> 4.0K	3.0-3.9K	2.0-2.9K	1.0-1.9K	< 1K
Platelets	WNL	75.0K-WNL	50-74.9K	25.0-49.9K	< 25K
Hemoglobin	WNL	10.0g-WNL	8.0-10.0g	6.5-7.9g	< 6.5g
Neutrophils	> 2.0K	1.5-1.9K	1.0-1.4K	0.5-0.9K	< 0.5K
Lymphocytes	> 2.0K	1.5-1.9K	1.0-1.4K	0.5-0.9K	< 0.5K
Hemorrhage, Clinical	None	Mild, No Transfusions	Gross, 1-2 U PRBC	Gross, 3-4 U PRBC	Massive, > 4 U PRBC
Infection	None	Mild	Moderate	Severe	Life-Threatening

Gastrointestinal					
Nausea	None	Able to Eat	Intake Decreased	No Significant Intake	
Vomiting	None	1x/24 hours	2-5x/24 hours	6-10x/24 hrs	> 10x/24 hrs
Diarrhea	None	Increase of 2-3x/24 hours	Increase of 4-6x/24 hours	Increase of 7-9x/24 hours	Increase of >10x/24 hrs
Stomatitis	None	Painless Ulcers	Painful Ulcers, Can Eat	Painful Ulcers, Cannot Eat	Requires IV Nutrition
Hepatic					
Bilirubin	WNL		< 1.5x WNL	1.5-3.0x WNL	> 3x WNL
SGOT/SGPT	WNL	< 2.5x WNL	2.6-5.0x WNL	5.1-20x WNL	>20x WNL
Alk Phos	WNL	< 2.5x WNL	2.6-5.0x WNL	5.1-20x WNL	>20x WNL
Liver/Clinical	No Change			Precoma	Hepatic Coma
Kidney/Bladder					
Creatinine	WNL	< 1.5x WNL	1.5-3.0x WNL	3.1-6.0x WNL	> 6.0x WNL
Proteinuria	No Change	1+ < 0.3 gm%	2-3+ 0.3-1.0 gm%	4+ > 1.0 gm%	Nephrotic Syndrome
Hematuria	Negative	Microscopic	Gross	With Clots	Transfusion
Alopecia	No Loss	Mild	Total		
Cardiovascular					
Dysrhythmia	None	Asymptomatic No Therapy	Persistent No Therapy	Requires Therapy	Hypotension, V-tach/V-fib
Cardiac	None	Decline of EF by < 20%	Decline of EF by > 20%	Mild CHF, Rx Responsive	Refractory CHF
Ischemia	None	Nonspecific ST-T Wave changes	Asymptomatic Ischemic changes	Angina, No Infarction	Acute MI
Pericardial	None	Asymptomatic Effusion	Pericarditis, rub, EKG changes	Symptomatic Effusion	Tamponade
Hypertension	None	Transient, >20mm Hg	Persistent, > 20 mm, No Rx	Requires Therapy	Hypertensive Crisis
Hypotension	None	Transient, No Therapy	Fluid Replacement	Hospitalized < 48 Hours	Hospitalized > 48 Hours
Pulmonary	No Change	Asymptomatic Abnormal PFT	Dyspnea on Exertion	Dyspnea, no exertion	Dyspnea at Rest
Toxicity Grade	**0**	**1**	**2**	**3**	**4**
Neurologic					
Neuro-sensory	No Change	Mild Paresthesia	Moderate Sensory Loss	Severe Loss, Symptomatic	
Neuro-motor	No Change	Subjective Weakness	Mild Objective Weakness	Impairment of Function	Paralysis
Cortical	None	Mild Somnolence, Agitation	Moderate Somnolence, Agitation	Severe, with Confusion or Hallucination	Coma or Seizures

Cerebellar	None	Slight Change Coordination	Speech Slur Tremor, Nystagmus	Ataxia	Cerebellar Necrosis
Mood	No Change	Mild Anxiety or Depression	Moderate	Severe	Suicidal
Headache	None	Mild	Transient, Moderate-Severe	Unrelenting, Severe	
Constipation	None	Mild	Moderate	Severe	Ileus >96 Hrs
Hearing	No Change	Asymptomatic Audiometry changes	Tinnitus	Correctable Loss	Deaf, not Correctable
Vision	No Change			Symptomatic Subtotal Loss	Blindness
Skin	No Change	Macular/ Papular Rash, Asymptomatic	Rash with Pruritus	Generalized Eruption	Exfoliative or Ulcerative Rash
Allergy	None	Transient Rash, Temp<38□C	Urticaria, Mild Broncho-spasm,T>38□	Serum Sickness, Bronchospasm	Anaphylaxis
Fever	None	37.1-38□C	38.1-40□ C	>40□,<24 Hrs	>40□, >24 Hrs
Local	None	Pain	Inflammation Phlebitis	Ulceration	Plastic Surgery Rx
Weight Change	< 5%	5-9.9%	10-19.9%	> 20%	

Metabolic

Hyper-Glycemia	< 116	116-160	161-250	251-500	> 500, Ketoacidosis
Hypoglycemia	> 64	55-64	40-54	30-39	< 30
Amylase	WNL	< 1.5x WNL	1.5-2.0x WNL	2.1-5.0x WNL	>5.1x WNL
Hyper-Calcemia	< 10.6	10.6-11.5	11.6-12.5	12.6-13.5	> 13.5
Hypocalcemia	> 8.4	8.4-7.8	7.7-7.0	6.9-6.1	< 6.0
Hypo-Magnesemia	> 1.4	1.4-1.2	1.1-0.9	0.8-0.6	< 0.5

Coagulation

Fibrinogen	WNL	.75-1x WNL	.5-.74x WNL	.25-.49x WNL	> 24x WNL
PT	WNL	1-1.25x WNL	1.26-1.5x WNL	1.51-2.0x WNL	> 2.0x WNL
PTT	WNL	1-1.25x WNL	1.26-1.5x WNL	1.51-2.0x WNL	> 2.0x WNL

APPENDIX III
Vitamin D Analogues

Analogue	Manufacturer	Code Name	Relative Competitve index for VDR (Calcitriol=100)	Dosing info	Comments
EB 1089 (24a,26a,27a,-Trihomo-22,24-diene-1□a,25-$(OH)_2$-D_3)	Leo Pharmaceutical	IC	17 (chick)	0.1 - 0.5 mcg/kg qd	
KH 1060 (20-Epi-22-oxa-24a,26a,27a-trihomo-1□,25-$(OH)_2$-D_3)	Leo Pharmaceutical	ID	25 (chick) 120 (chick)		CI = 1.3
MC 1288 (1,25$(OH)_2$-20-epi-D_3)	Leo Pharmaceuticals	IE	147 (chick) 120 (chick)		
MC 903 (Calcipotriol) (1□24S-$(OH)_2$-22-Ene-26,27-dehydro-D_3)	Leo Pharmaceutical	BT	111 (chick) 240 (U-937 cells) 76 (chick) 100 (chick)	Rats - 50 mcg/kg qod	Topical form used in human breast cancer trial, approved as antipsoriatic CI < 0.01
1,25-(OH)2-16-Ene-D3	Roche Pharmaceutical	HM	165 (chick) 240 (rat intestine) 250 (rat intestine)		
1,25-(OH)2-16-Ene-23-yne-D_3	Roche Pharmaceutical	V	68 (chick) 70 (chick) 90 (chick) 50 (rat intestine) 80 (chick)	Mice - 0.5 mcg 3 x/week, 1.6 mcg qod	
25-(OH)2-16-Ene-23-yne-D_3	Roche Pharmaceutical	AT	0.4 (chick) < 0.4 (chick) 0.07 (rat intestine)		
22-Oxacalcitriol (22-Oxa-1□,25-$(OH)_2$-D_3)	Chugai Pharmaceutical	EU	15 (chick) 12 (chick) 100 (rat osteosarcoma) 7 (chick) 100 (MCF-7 cells)		CI < 0.001
1 □ (OH) D_5	University of Illinois	-	N/A		
ZK 161422 (20-methyl-(1,25$(OH)_2$D3	Institute of Medical Chemistry – Schering AG	-	N/A		
ZK 157202 (20-methyl-23-ene(1,25$(OH)_2$D 3	Institute of Medical Chemistry – Schering AG	-	N/A		
1alpha-(OH)-D3		BP	0.17 (chick)		

We claim:

1. A method for the treatment of a hyperproliferative disease in a subject, wherein the hyperproliferative disease responds to treatment with a Vitamin D drug, comprising administering to the subject a therapeutically effective pulse dose of the Vitamin D drug in a sufficient amount to have an antiproliferative effect, without inducing severe symptomatic hypercalcemia.

2. The method of claim 1, comprising administering the Vitamin D drug to a subject having a neoplasm that expresses a Vitamin D receptor.

3. The method of claim 2, wherein the neoplasm is selected from the group of cancer of the prostate, breast, colon, lung, head and neck, pancreas, endometrium, bladder, cervix, ovaries, squamous cell carcinoma, renal cell carcinoma, myeloid and lymphocytic leukemia, lymphoma, medullary thryoid carcinoma, melanoma, multiple myeloma, retinoblastoma, and sarcomas of the soft tissues and bone.

4. The method of claim 3, wherein the neoplasm is breast cancer or prostate cancer.

5. The method of claim 1, wherein the Vitamin D drug has a calcemic index greater than or equal to calcipitriol.

6. The method of claim 1, wherein the Vitamin D drug has a calcemic index greater than 1.0.

7. The method of claim 5, wherein the Vitamin D drug has a half-life that is no greater than about 1 day.

8. The method of claim 7, wherein the Vitamin D drug has a half-life that is no greater than about 6 hours.

9. The method of claim 8, wherein the Vitamin D drug is administered in an amount that raises a serum level of Vitamin D in the subject with a tumor to a supraphysiologic amount for a sufficient period of time to induce differentiation or regression of the tumor without causing symptomatic hypercalcemia.

10. The method of claim 1, wherein the Vitamin D drug is calcitriol, which is administered in a therapeutically effective pulse dose no more than once every three days.

11. The method of claim 10, wherein the calcitriol is administered orally in a dose of at least 0.12 mcg/kg per day no more than once per week.

12. The method of claim 10, wherein the calcitriol is administered orally in a dose of at least 0.48 mcg/kg or about 1 mcg/kg per day no more than once per week.

13. A method of treating a tumor in a subject, wherein the tumor expresses a Vitamin D receptor and is responsive to treatment with a Vitamin D drug, the method comprising administering orally to the subject, no more than once every three days, a dose of calcitriol of about 0.5 mcg/kg.

14. The method of claim 13, wherein the Vitamin D drug is administered to the subject no more than once per week.

15. A composition comprising a Vitamin D drug in a pharmaceutical dosage form in a dosage that would cause hypercalcemia is administered daily.

16. The composition of claim 15, wherein the Vitamin D drug is calcitriol, contained in the composition in an amount of at least 5 mcg.

17. The composition of claim 16, wherein the pharmaceutical dosage form is an oral dosage form containing at least 100 mcg per unit of the oral dosage form.

18. The composition of claim 16, wherein the pharmaceutical dosage form is a tablet or capsule.

19. The composition of claim 16, wherein the Vitamin D drug is calcitriol, and the pharmaceutical dosage form is a tablet containing at least 5 mcg calcitriol.

20. The method of claim 1, wherein the subject is prescribed a reduced calcium diet for a sufficient period of time prior to administration of the Vitamin D drug to reduce absorption of dietary calcium.

21. A method of treating in a subject a tumor that expresses a Vitamin D receptor, the method comprising raising a blood level of Vitamin D to a sufficiently supraphysiologic level for a sufficient period of time to inhibit growth of the tumor, without inducing hypercalcemia in the subject.

22. The method of claim 21, wherein the blood level of Vitamin D is raised by administering a Vitamin D drug to the subject.

23. The method of claim 22, wherein the Vitamin D drug is calcitriol.

24. The method of claim 23, wherein the calcitriol is administered in a dose of about 0.50 mcg/kg once per week.

AMENDED CLAIMS

[received by the International Bureau on 21 July 1999 (21.07.99); original claim 15 amended; remaining claims unchanged (1 page)]

15. A composition comprising a Vitamin D drug in a pharmaceutical dosage form in a dosage that would cause hypercalcemia if administered daily.

16. The composition of claim 15, wherein the Vitamin D drug is calcitriol, contained in the composition in an amount of at least 5 mcg.

17. The composition of claim 16, wherein the pharmaceutical dosage form is an oral dosage form containing at least 100 mcg per unit of the oral dosage form.

18. The composition of claim 16, wherein the pharmaceutical dosage form is a tablet or capsule.

19. The composition of claim 16, wherein the Vitamin D drug is calcitriol, and the pharmaceutical dosage form is a tablet containing at least 5 mcg calcitriol.

20. The method of claim 1, wherein the subject is prescribed a reduced calcium diet for a sufficient period of time prior to administration of the Vitamin D drug to reduce absorption of dietary calcium.

21. A method of treating in a subject a tumor that expresses a Vitamin D receptor, the method comprising raising a blood level of Vitamin D to a sufficiently supraphysiologic level for a sufficient period of time to inhibit growth of the tumor, without inducing hypercalcemia in the subject.

22. The method of claim 21, wherein the blood level of Vitamin D is raised by administering a Vitamin D drug to the subject.

23. The method of claim 22, wherein the Vitamin D drug is calcitriol.

24. The method of claim 23, wherein the calcitriol is administered in a dose of about 0.50 mcg/kg once per week.

STATEMENT UNDER ARTICLE 19(1)

In the International Search Report dated 24 May 1999, claims 15-19 were said to lack novelty (Category X) in view of Miller et al. (Clin. Cancer Res., 1995, 1(9), 997-1003), Welsh (Biochem. Cell Biol., 1994, 72(11&12), 537-545), or Buras et al. (Breast Cancer Res. Treat., 1994, 31(2/3), 191-202). All three of these publications disclose *in vitro* tests aimed at identifying cells that express Vitamin D receptors and are potentially susceptible to treatment with Vitamin D or its analogs. These publications do not address the use of Vitamin D drugs *in vivo*. The claims of the present application, in contrast, are directed at pulsed *in vivo* administration of antineoplastic quantities of Vitamin D drugs. Claims 15-19 call for pharmaceutical compositions having dosage amounts of Vitamin D drugs that would cause hypercalcemia if administered daily. None of the cited references disclose pharmaceutical compositions containing the dosages of Claim 15, much less do they disclose the oral or tablet forms of Claims 17-19.

The Search Report also cited Miller et al., Welsh, and Buras et al. as negating an inventive step (Category Y) for claims 1-14 and 20-24. Claims 1-14 and 20-24 call for a method of administering a Vitamin D drug *in vivo*, in therapeutically sufficient amounts, without inducing hypercalcemia. Certain claims (such as Claim 10) are directed to pulsed administration of the drug using regimens that avoid hypercalcemia *in vivo*, while surprisingly exerting an antiproliferative or antineoplastic effect. The three publications cited against these claims all describe *in vitro* testing of cancer cells for potential vitamin D drug susceptibility.

Welsh explicitly recognizes the limitation that the calcemic activity of Vitamin D drugs imposes on their *in vivo* use, but does not offer any suggestion as to how to overcome this limitation. (Welsh, p 539). Miller et al. teaches that cell lines which metabolize 1α,25 dihydroxyvitamin D_3 quickly, are less affected by that substance. (Miller et al., p. 1003). Contrary to this teaching, Claims 7-8 call for vitamin D drugs with short half-lives in order to prevent symptomatic hypercalcemia. Buras et. al. suggest in their publication that Vitamin D drugs may be most effective for the **prevention** of breast cancer. The present claims, however, are directed at the **treatment** of hyperproliferative diseases such as breast cancer. Since Miller et al., Welsh, and Buras et al. do not disclose or suggest pulsed doses of vitamin D analogs, which achieve transient supraphysiological *in vivo* concentrations, these publications do not support a rejection of the claims of the present application. Moreover, the unexpected finding that intermittent doses of Vitamin D and its analogs (as opposed to sustained concentrations *in vitro*) exert an antiproliferative/antineoplastic action, also rebuts any contention that the claimed invention lacks an inventive step.

FIG 1

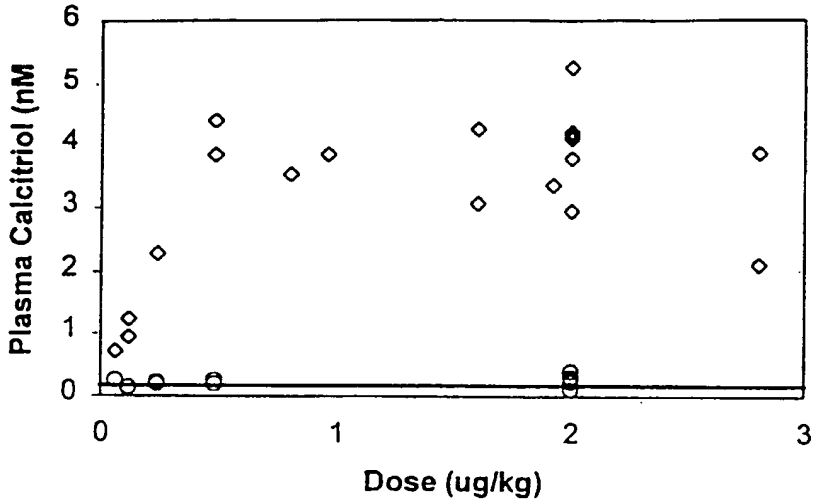
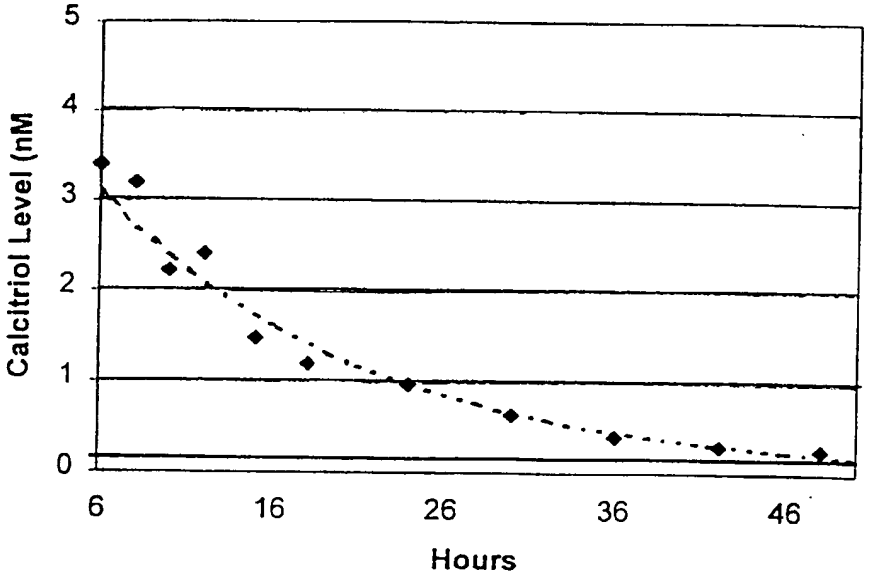

Plasma Calcitriol (nM
6
5
4
3
2
1
0
0
1
2
3
Dose (ug/kg)

FIG 2


Calcitriol Level (nM
5
4
3
2
1
0
6
16
26
36
46
Hours

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/06442

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/59
US CL :514/167

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/167

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, USPATFULL, HCAPLUS- calcitriol for the treatment of cancers especially of the breast or prostate.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X - Y	Database HCAPLUS on STN, American Chemical Society, AN 1995:870552, MILLER, G.J. et al. 'Vitamin D receptor expression, 24-hydroxylase activity, and inhibition of growth by 1.alpha.,25-dihydroxyvitamin D3 in seven human prostatic carcinoma cell lines,' abstract, Clin. Cancer Res., 1995, 1(9), 997-1003.	15-19 ------ 1-14 and 20-24
X - Y	Database HCAPLUS on STN, American Chemical Society, AN 1995:610296, WELSH, J. 'Induction of apoptosis in breast cancer cells in response to vitamin D and antiestrogens,' abstract, Biochem. Cell Biol., 1994, 72(11&12), 537-545.	15-19 -------- 1-14 and 20-24

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"B"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
09 MAY 1999	24 MAY 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer M. MOEZIE Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/06442

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X - Y	Database HCAPLUS on STN, American Chemical Society, AN 1995:222679, BURAS, R.R. et al. 'Vitamin D receptors in breast cancer cells,' abstract, Breast Cancer Res.Treat., 1994, 31(2/3), 191-202.	15-19 -------- 1-14 and 20-24